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A pilot study on using biochars as sustainable amendments to inhibit rice uptake of Hg from a historically polluted soil in a Karst region of China

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ABSTRACT

We studied the addition of two biochars (rice shell biochar (RSB) and wheat straw biochar (WSB)) to soil at doses of 24–72 t/ha on the dynamics of pH, dissolved organic carbon (DOC), sulfate, Fe(III), and Fe(II), as well as on mercury (Hg) mobility in the pore water of a polluted paddy soil, throughout the rice-growing season. The effect of biochar addition to soil on rice biomass and Hg accumulation was also investigated. The key results showed that the addition of RSB or WSB to soil improved significantly the biomass of aboveground tissues of rice plants, particularly at higher dose treatments, compared with the control. The RSB treatment noticeably decreased Hg concentration in the pore water compared to the control, throughout the rice-growing season, and this decrease was likely due to the decreased Hg mobility by the RSB by promoting the level of sulfate in the pore water, which might be reduced to sulfide to combine with Hg to form Hg sulfides. The extent of Hg concentration reduction in the pore water was less pronounced in the WSB treatments relative to the RSB treatments. Addition of RSB to soil at doses of 24–72 t/ha decreased significantly Hg contents in the stalk, bran, hull and polish rice of rice plants compared to the non-treated rice (control), particularly Hg content in the polished rice was below the Chinese safety level ($< 20\,\mathrm{ng\,g^{-1}}$, GB2762-2012). The WSB treatments showed limited effects on rice tissues Hg. Biochar (RSB) may offer a promising method for managing the risk of Hg in paddy field by inhibiting rice Hg uptake.

1. Introduction

Soil pollution accelerates land degradation, and thus there is a high demand for polluted soil remediation (Wang et al., 2018a). Mercury and its compounds have serious toxic effects on human reproductive, immune, and digestive systems and are among the top 10 pollutants associated with major public health issues according to the World Health Organization (WHO) (Rothenberg and Feng, 2012; Wang et al., 2012a). Anthropogenic activities (e.g., coal combustion, artisanal and small-scale gold mining activities, and non-ferrous metal refining activities) release large amounts of Hg-containing waste products to the surrounding environments, causing extensive Hg pollution (Wang et al., 2012b). Countries in Southeast Asia, such as China, Indonesia, and Malaysia, face an urgent Hg pollution problem due to extensive mining and retorting activities and the widespread production and use of Hg-containing products in this region over the last century (Li et al., 2009).

Pollution of paddy fields with Hg receives considerable public concerns, owing to the tendency of Hg methylation by bacteria in these sites, and the methylated Hg (MeHg) is highly bioavailable to rice seedlings (Rothenberg and Feng, 2012). It is reported that Hg-laden rice constitutes a significant proportion of the dietary intake of Hg among inhabitants of inland China (Zhang et al., 2010a, 2010b), particularly within Hg mining regions, where rice consumption represents nearly 94–96%, and 19–24% of the daily MeHg and inorganic Hg (IHg) intake, respectively (Li et al., 2015; Zhang et al., 2010a, 2010b). Social concern regarding Hg-polluted rice is increasing because, along with fish consumption, the consumption of Hg-containing rice is a common pathway for Hg intake in Asia (Rothenberg et al., 2014).

Development of sustainable and cost-effective methods to remediate Hg-polluted soils is particularly important (Wang et al., 2019). Due to its effectiveness and large-scale applicability, in situ immobilization of Hg in the soil constitutes a good approach to reducing Hg accumulation in crops (Cundy et al., 2013, 2016; Li et al., 2018; Wang et al., 2018b). The chemical agents, including selenate/selenite (Tang et al., 2017),

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sulfur (Wang et al., 2016), have been tested for Hg-polluted paddy fields, but their large-scale applications seem to be sparse, perhaps due to the potential risk to soils by introducing these chemical agents. For instance, the over-application of selenium may lead to the pollution of soil with this metal. Therefore, there is a growing interest in developing sustainable, and environmentally friendly materials for Hg remediation (Wang et al., 2017).

Recently, researchers have become particularly interested in biochar (Shu et al., 2016), which is produced by the high-temperature pyrolysis of biomass under minimal oxygen supply (Woolf et al., 2010). It is alkaline, and has a high specific surface area and abundance of functional groups (Gomez-Eyles et al., 2013). Addition of biochar to soils may lead to the decrease of toxic elements mobility, as well as provide additional benefits of improving crop productivity and increasing the soil carbon pool (El-Naggar et al., 2018; Kong et al., 2011; Park et al., 2011). Biochar is able to remove Hg^{2+} from solutions (Boutsika et al., 2013), and the underlying mechanism by which biochar immobilizes Hg is attributable to the coordination of Hg with the hydroxyl, carboxyl, and sulfur groups; the Hg-π binding induced by the π electrons of the $C=C$ and $C=O$ bonds (Liu et al., 2016; Xu et al., 2016); and the graphite-like structure of biochar (Dong et al., 2013).

Although many studies have been carried out for Hg immobilization using biochar, its effect on the behaviour of Hg in paddy fields and rice uptake of Hg is poorly understood. Unlike sediments, paddy fields are seasonally covered by rice seedling and its growth causes the dynamic of a variety of biogeochemical factors such as pH, SO_4^{2-}, Fe(II), Fe(III), and dissolved organic carbon (DOC), which affect the behaviour of metals significantly (Beckers and Rinklebe, 2017; Benoit et al., 1999; Sunderland et al., 2006). The integrated effect of biochar and rice plant growth on the dynamic of pH, SO_4^{2-}, Fe(II), Fe(III), and DOC, and on Hg mobility and rice uptake of Hg remain unknown.

To address this knowledge gap, we added two biochars RSB and WSB, which were made by pyrolysis of the rice hull (RSB) and wheat straw (WSB) with limited oxygen supply at 480–660 ℃ and 350–450 ℃, respectively, to Hg-polluted soil collected from a historically polluted paddy field (total $Hg = 129 \pm 24\,mg\,kg^{-1}$) in a Karst region of China, and investigated the dynamic of pH, SO_4^{2-}, Fe(II), Fe(III), DOC, and mobile Hg in the pore waters of these biochar-treated soils throughout the rice-growing season, and compared these to the non-treated control. Also, the impact of biochar addition to soil on rice plant growth and Hg uptake were investigated. The specific aims of this pilot study were: (1) to quantify the effect of biochars addition to soils on the growth of rice plants; (2) to investigate biochars addition to soil on the dynamic of dissolved organic carbon (DOC), pH, sulfate, Fe(II), Fe(III), and Hg, in the pore water of soils throughout the rice-growing season; (3) to explore biochars addition to soil on Hg accumulation in the rice plant tissues; and (4) to reveal the potential mechanism by which biochar affects Hg mobility in the paddy soil.

2. Material and methods

2.1. Biochar production

Two commercially produced biochars RSB and WSB (purchased from the Beijing HL Biological Co., LTD. (Beijing, China)), which were made by pyrolysis of the rice hull (RSB) and wheat straw (WSB) with limited oxygen supply at 480–660 ℃ and 350–450 ℃, respectively, were used. They were sieved through a 4-mm nylon mesh prior to use.

2.2. Experimental design

Mercury polluted soil was collected from a farmland at Wanshan Hg mine in China. Detailed soil sampling information is given in S1.1. A series of pre-cleaned 5-L plastic pots were set in a field and each pot was filled with approximately 3.6 kg of Hg-polluted soil (Fig S1-A). The RSB or WSB was applied at a dose of 24 t of biochar per hectare (RSB/WSB 24 t/ha), and 72 t of biochar per hectare (RSB/WSB 72 t/ha), similar to the doses used by Chan et al. (2007). A group of pots without biochar treatment was designated as control. Each treatment group included three replicates. The biochar and soil were thoroughly homogenized prior to filling the pot. To maximally homogenize the biochars and soils, we put them into a plastic bag (10 L), and manually shaken the bag for 10 min for each treatment. All pots were arranged by a randomised design method. Prior to transplanting, the soil was flooded with purified water (total $Hg < 0.02\,ng\,L^{-1}$) produced by a water purified machine (HLDRO-1T-30H/T, Guangxi VOLARDDA Co., China) to a depth of 3 cm and equilibrated for 5 days. Three pots filled with non-polluted soil (total $Hg = 0.3\,mg\,kg^{-1}$; bioavailable $Hg = 0.021\,ng\,L^{-1}$) were used to monitor the atmospheric Hg deposition on the leaf of the rice plant at the experimental site.

The 30-day-old rice seedlings (*Oryza sativa* L., cv. II-406) provided by the Guizhou Rice Research Institute were transplanted on 25 May 2015 and harvested on 24 September 2015. Purified water was regularly supplied throughout the whole growing season. The pots were kept flooded throughout the growth period, except for days 110–115, during which all pots were drained to promote greater rice yield. About 200 mg, 250 mg, and 200 mg Urea (CON_2H_4) was applied to each pot before transplanting, at the tillering stage, and at the panicle stage, respectively. Additionally, about 560 mg nitrogen-phosphate-potassium compound fertilizers (15% N-42% P-5% K (w/w)) were applied to each pot both at the heading and filling stages. The fertilization, irrigation, and pesticide application rates were kept consistent for all the pots during the experiment.

2.3. Sample collection and analysis

The basic physical-chemical properties of the soil, including pH, total nitrogen, total carbon, total sulfur, particle size distribution, total Hg, and organic matter content, were determined using the methods described by Wang et al. (2012a). The pore water in each pot was sampled on days 5, 33, 52, 71, 81, 103, and 118 during the rice-growing season using a passive pore water sampler (Daxiong Monitor Co., Shengzheng, China) with a pore size of 10-µm (Fig S1-B). By using this passive pore water sampling, we were able to collect approximately 200–300 mL of pore water from each pot. Pore water samples were divided into six subgroups for total Fe, ferrous Fe, pH, SO_4^{2-}, dissolved organic carbon (DOC), and total Hg analyses. The Fe(II) and total Fe concentrations were analysed for the samples collected on days 33, 52, 71, 81, 103, and 118. Detailed analytical protocol information is given in S1.2.

The rice plants were divided into root, stalk, leaf, and panicle sections by stainless-steel scissors prior to being cleaned. Root sections were washed with 0.01 M ethylenediaminetetraacetic acid (EDTA) and purified water, while the aboveground tissues (root, stalk, leaf, and panicle sections) were cleaned with purified water only. All the tissues were air-dried. The air-dried panicle was further divided into the hull, bran, and polished rice. All plant materials were ground to powders using an electrical micro-crusher (IKA®-Werke GmbH & Co., Staufen, Germany), which were carefully cleaned after processing each sample to avoid any cross-contamination during the sample pre-treatment. Plant powders were kept in Ziploc bags and stored in a refrigerator at + 4 ℃ in the dark. The analytical methods used to determine the plant biomass and biochar parameters are given in S1.3 and S1.4 respectively.

2.4. Data quality control and assurance

Standard reference materials, GBW 070009 (soil) and GBW10020 (plant), purchased from the Institute of Geophysical and Geochemical Exploration, China, were digested in triplicates for data quality control and assurance purposes. Mercury recovery ratios were 89–105% for the soil and 90–100% for the plant. The relative percentage differences between replicates for the soil and plant samples were < 9% and < 5%, respectively.

2.5. Data analysis

Origin 8.0 software (Origin Lab Co., USA) was used to plot the data. Statistical analysis was performed using *SPSS* 17.0 software (SPSS Inc., USA). One-way analysis of variance (ANOVA) was used to test for significant differences between the treatment groups. The Pearson correlation analysis was applied to examine the associations of Hg with the different rice plant tissues and the geochemical variables in the pore water. Significant differences between the treatment groups are denoted by different lower-case characters or asterisks (*) in the figures and tables.

3. Results and discussion

3.1. The physico-chemical properties of biochar and soil, and plant biomass

The general physicochemical properties of the studied soil are shown in Table S1. It was calcareous and slightly acidic, with total carbon, nitrogen, sulfur, and organic matter contents of 20, 2.1, 1.0, and 40 mg g^{-1}, respectively. Its texture was characterized as sandy loam. The total Hg content was 129 mg kg^{-1}, which is two orders of magnitude over the threshold content of 1.5 mg kg^{-1} recommended by the Chinese government for farmland (GB1568-1995) (CNEPA, 1995). The WSB was more alkaline and had a lower total carbon, total sulfur, and sulfate content than the RSB. The presence of higher sulfur content in the RSB relative to the WSB could be due to the enrichment of sulfur in the former's feedstock compared to the latter's. The WSB had a higher DOC content than the RSB, indicating a larger labile carbon pool in the former than the latter. The difference in DOC content between two biochars might be related to the pyrolysis temperatures. Prior studies reported that the production of biochar with high pyrolysis temperature led to the increase in aromaticity and aromatic condensation of carbon in biochar, which were considered to be more stable than the nonaromatic carbon in the soils (McBeath et al., 2011; Nguyen et al., 2010). The RSB was pyrolysed at 480–660 °C, while the WSB was at 350–450 °C. It is thus likely that the carbon in the RSB was more stable than that in the WSB.

The differences in root weight, length, and volume between the control and biochar (both RSB and WSB) treatments were statistically insignificant ($P > 0.05$) (Table 1), which is in agreement with previous studies, reporting that the application of rice husk-biochar had little effects on root growth under potted conditions (Noguera et al., 2010; Shinogi, 2016). The limited effect of biochar on root development in rice is perhaps due to the limited size of pot (Fig S1-C), which hinders the further root development. Unlike root, application of RSB or WSB increased significantly ($P < 0.05$) both the stem and leaf weight compared with the control plants, particularly at higher dosage treatment (Table 1). Moreover, the stem of rice grown in RSB treated soil was noticeably longer ($P < 0.05$) than that of the non-treated control. The enhancement of rice growth by biochars was primarily due to their abilities to increase the availability of nutrients and improve the soil quality, as documented by prior studies (Cui et al., 2011; Dong et al., 2015; Steiner et al., 2008). A recent mechanic study by Hagemann et al. (2017) showed that the enhanced soil nutrient availability by biochar was induced by its surface-covered organic coating, which promoted biochar's imesoporosity, hydrophilicity, and redox-active moieties.

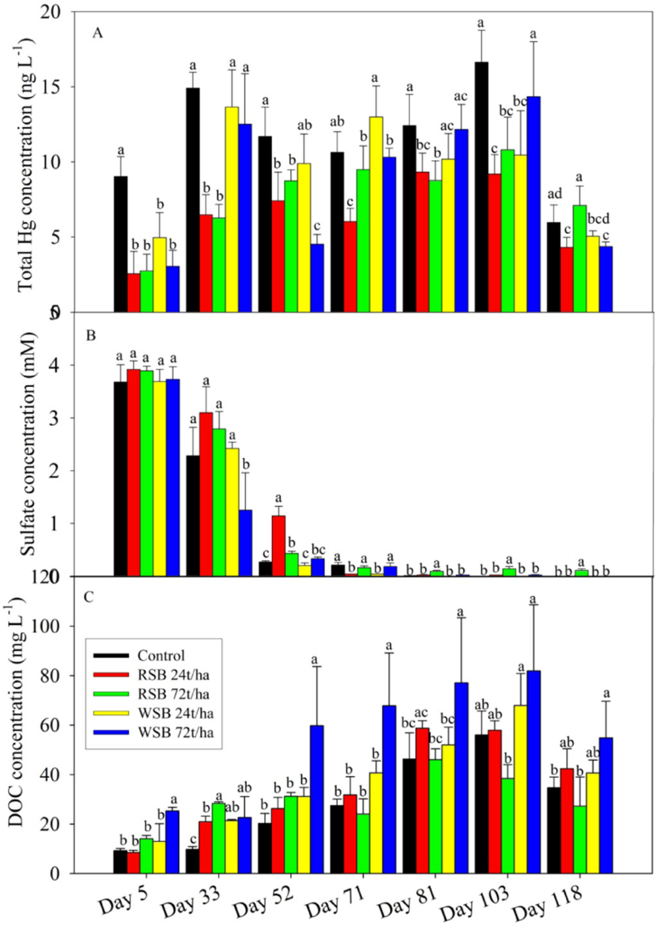

Fig. 1. Bar charts for the total Hg (A), sulfate (B), and dissolved organic carbon (DOC) concentrations, in the pore water for each treatment. The different lower-case character indicates that the differences in total Hg, sulfate, and DOC concentrations for the five treatments are significant at $\boldsymbol{P} < 0.05$. The error bar represents the standard deviation of the three replicates for each treatment ($n = 3$).

3.2. Mercury and biogeochemical parameters in the pore water

Throughout the rice-growing season, the concentration of Hg in the pore water ranged from 5.95 to 16.63 ng L^{-1}, 2.6 to 9.3 ng L^{-1}, 2.8 to 10.8 ng L^{-1}, 5.0 to 13.7 ng L^{-1}, and 3.0 to 14.3 ng L^{-1} for the control, 24-t/ha RSB, 72-t/ha RSB, 24-t/ha WSB, and 72-t/ha WSB groups, respectively (Fig. 1-A). These data are close to the values reported by Rothenberg and Feng (2012), who found Hg concentrations of 9.9–720 ng L^{-1} in the pore water of a heavily polluted soil at Wanshan

Table 1
The root length, root weight, root volume, stem length, stem weight and leaf weight of rice plants. Mean ± sd ($n = 3$).

	Root weight (g)	Root volume (cm^3)	Root length (cm)	Stalk length (cm)	Stalk weight (g)	Leaf weight (g)
Control	13.4 ± 2.2 a	114 ± 17 a	39.2 ± 1.1 a	92 ± 2.4 a	51.5 ± 2.4 a	11.8 ± 2.2 a
RSB 24 t/ha	13.6 ± 1.2 a	122 ± 3.5 a	43 ± 3.5 a	99 ± 2.6 b	54.8 ± 3.7 a	12.6 ± 1.2 a
RSB 72 t/ha	16.2 ± 2.5 a	124 ± 8.5 a	35.8 ± 4.9 a	101 ± 3.3 b	77.5 ± 6.3 b	21.0 ± 1.9 b
WSB 24 t/ha	11.6 ± 2.1 a	99 ± 26.9 a	41.8 ± 1.1 a	92 ± 2.8 a	57.1 ± 0.9 a	14.3 ± 1.0 a
WSB 72 t/ha	15.3 ± 1.0 a	106 ± 13 a	43.5 ± 0.7 a	95 ± 0.5 a	62.2 ± 3.6 b	18.4 ± 1.3 b

The different lower-case character means each plant biomass parameter was statistically different ($P < 0.05$) between the control and biochar treatments.

Hg mine in China. The average concentration of Hg in the pore water of 24-t/ha RSB, 72-t/ha RSB, 24-t/ha WSB, and 72-t/ha WSB treatment throughout the rice-growing season was decreased by 44%, 34%, 17%, and 26%, respectively, compared to the control, particularly in RSB treatments where Hg concentration in the pore water from each sampling (except on day 118) was significantly lower than that of control (Fig. 1-A). Whereas, Hg concentration in the pore water of the WSB treatment varied with the sampling time, compared to the control. It appears that RSB was more efficient than WSB at reducing Hg mobility in the pore water in our soils. The sharply decrease in the Hg concentration on day 118 could be due to the drainage of soil, a common paddy field management protocol used to improve the rice grain yield in the world (Tabuchi, 2004).

Sulfate concentrations ranged from 0.003 to 3.7 mM, 0.004 to 3.9 mM, 0.1 to 3.9 mM, 0.002 to 3.7 mM, and 0.004 in 3.7 mM in the control, 24-t/ha RSB, 72-t/ha RSB, 24-t/ha WSB, and 72-t/ha WSB soils, respectively. Sulfate concentration sharply decreased on day 33 for all five treatments (Fig. 1-B), which might be resulted from the sulfate reduction by sulfate-reducing bacteria (SRB) (Pester et al., 2012). Throughout the rice-growing season, the average sulfate concentration for the 24-t/ha RSB treatment (1.2 mM) and the 72-t/ha RSB treatment (1.1 mM) was 15–39% higher than that of the control (0.9 mM), 24-t/ha WSB (0.91 mM), and 72-t/ha WSB (0.80 mM) treatments. The elevated sulfate concentration in the soil of RSB treatments relative to that of WSB treatments might be mainly due to the introduction of more sulfate to soils by the RSB than the WSB (Table S1).

The concentration of DOC gradually increased throughout the rice-growing season (Fig. 1-C), likely by the enhanced organic matter decomposition by microbe (Lu et al., 2002), as root exudates could promote microbe activities through providing carbon energy sources (Shen et al., 2015). The pots treated with 72-t/ha WSB showed the highest concentrations of DOC ($P < 0.05$) throughout the rice-growing season (except for day 33), while the control, RSB 24-t/ha, and WSB 24-t/ha treatments displayed similar levels of DOC. The RSB 24-t/ha treatment showed the lower concentration of DOC compared to the other treatments. The elevated DOC levels in the soil at higher treatment dosage of WSB compared to that of RSB might be resulted from the difference in the properties of two biochars. It has been reported that processing biochar at low temperatures (300–400 °C) stimulates carbon mineralization via the microbial decomposition of labile carbon, whereas processing biochar at high temperatures (525–650 °C) suppresses carbon mineralization in soil because of the relatively stable carbon within biochar (Sui et al., 2016; Zimmerman et al., 2011). The WSB was produced at a lower temperature and promoted a higher level of DOC than the RSB; thus, carbon mineralization might be more pronounced for the soil treated with the WSB. Moreover, the content of organic carbon of WSB was twice compared to that of RSB, its higher application dosage might result in more organic carbon in the soil.

Although both the WSB and RSB were more alkaline than the soil, no significant difference in the pH levels between the control and biochar treated soils was observed throughout the rice-growing season (except for days 5 and 33) (Fig S2-A), possibly due to the relatively strong pH buffer capacity of our calcareous soil (Robertson et al., 2012).

Both the RSB and WSB treatments resulted in significantly higher Fe (II) and Fe(III) concentrations in the pore water compared with the control throughout the rice-growing season (Fig S2,-B,-C), which indicates an promotive effect of the biochar on Fe oxides mobilization. It has been reported that addition of biochar to soil to effect Fe oxides mobilization mainly though its ability of enhancing the abundance of Fe(III) reducing bacteria including *Geobacter*, *Anaeromyxobacter*, *Desulfosporosinus*, and *Pedobacter* (Chen et al., 2016), and serving as electron shuttle between bacteria and Fe(III) minerals to promote microbial Fe oxides reduction (Jia et al., 2018; Kappler et al., 2014; Wang et al., 2017).

To better understand how the biogeochemical variables (DOC, pH, sulfate, Fe(II), and Fe(III)) affected the behaviour of Hg in the pore water, a Pearson correlation analysis was performed. As shown in Table 2, the poor correlation between Hg and Fe(II)/Fe(III)/pH indicates a relatively weak effect of these biogeochemical factors on the Hg mobility in the pore water. Mercury concentration was correlated positively with the DOC concentration, but negatively with the sulfate concentration (Table 2), indicating that the elevated DOC concentration might enhance Hg mobility, while the elevated sulfate concentration might reduce the mobility of this metal (Ravichandran, 2004; Ravichandran et al., 1998) in the pore water. It is well known that SRB utilize sulfate as a terminal electron acceptor, reducing it to sulfide (Barton and Fauque, 2009), which can readily combine with mercuric ion to form less bioavailable HgS complexes (Håkansson et al., 2008). It is possible that the presence of more sulfate might enhance the SRB activities to produce more sulfide to precipitate with Hg in the pore water in our biochar-treated soils. Dissolved organic carbon seems to promote the mobility of Hg (Ravichandran, 2004), likely by the solubilization of less bioavailable HgS by the break of Hg-S bond as a consequence of the change in the coordinative environment of Hg on the surface of HgS in the presence of DOC (Ravichandran et al., 1998; Waples et al., 2005).

Table 2
Pearson linear coefficients (*r*) matrix for THg, sulfate, Fe(II), Fe(III) and dissolved organic carbon (DOC) concentrations, as well as pH in the pore water. (THg, sulfate and DOC: $n = 105$; Fe(II) and Fe(III): $n = 90$).

	SO_4^{2-}	Fe(II)	Fe(III)	pH	DOC
THg	-0.34^{**}	ns	ns	ns	0.24^{*}
SO_4^{2-}		ns	-0.64^{**}	0.46^{*}	-0.62^{**}
Fe(II)			ns	ns	ns
Fe(III)				ns	0.59^{**}
pH					ns

*, **, significant levels at $P < 0.05$ and $P < 0.01$ respectively.
ns: not significant.

3.3. Mercury contents in the rice tissues

The Hg contents in the rice tissues were shown in Fig. 2. The Hg contents in the stalks of the RSB and WSB treatment groups were significantly lower ($P < 0.05$) relative to that of non-treated rice seedlings, which suggests that both biochars retarded Hg translocation to the shoot. A similar phenomenon has been reported for cadmium, zinc, and arsenic (Lin et al., 2017; Zheng et al., 2015). The "bio-dilution" effect, which refers to the decrease in the concentration of a pollutant with the increase in the biomass of an organism, contributes to the reduction of elements in biological tissues. We expected a minor role of this effect in our study due to the lack of an apparent relationship between the Hg content and the biomass of the plant (Figs. S3, S4). The extent of Hg reduction in the leaf was weaker than in the stalk. For instance, application of both types of biochar at a dose of 24-t/ha dramatically decreased the Hg content in the leaf, whereas no such effect was observed for the 72-t/ha treatment group, when compared to the control. Mercury in the leaves of rice seedlings grown in non-polluted soil should be partially sourced from atmospheric Hg° (Manceau et al., 2018). We detected Hg content of 201 $ng\ g^{-1}$ ($n = 3$) in the leaves of rice plants grown in non-polluted soil, indicating that a certain portion of the Hg in the leaf might be sourced from the atmosphere in our study. This may be explainable for the relatively weak effect of two biochars on leaf Hg, since they could not effect foliage Hg^0 uptake from the atmosphere.

Polished rice is the main edible part of the rice grain, and is the focus for Hg content concerns. According to the Chinese government, the safe Hg level for rice grains is 20 $ng\ g^{-1}$ (CNEPA, 2012). An average Hg level of 24 $ng\ g^{-1}$ in the non-treated control grain indicates the need to remediate the polluted soil. Application of RSB at treatment doses of 24- and 72-t/ha and of WSB at a treatment dose of 72-t/ha significantly

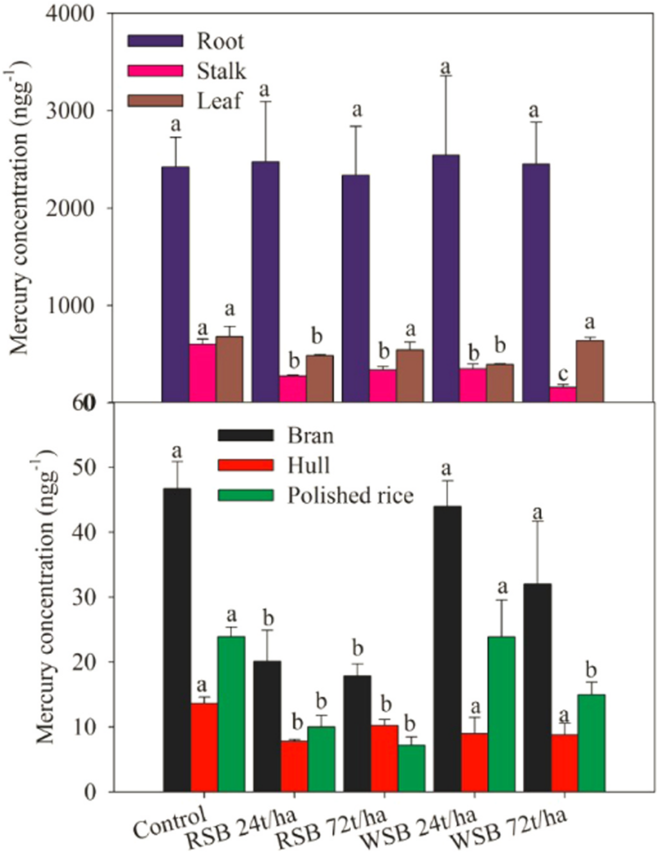

Fig. 2. Mercury contents in the polished rice, bran, hull, root, stalk, and leaf of rice plants. The different lower-case character in each figure indicates that the mean Hg content in the rice tissue differed significantly between the control and the RSB or WSB group. The error bar represents the standard deviation of the three replicates for each treatment ($n = 3$).

reduced ($P < 0.05$) the Hg contents in the polished rice (24 t/ha RSB: 10 ng g^{-1}; 72 t/ha RSB: 7.2 ng g^{-1}; 72 t/ha WSB: 15.0 ng g^{-1}) to a level below the Chinese standard (20 ng g^{-1}). Additionally, Hg contents were reduced in the bran by 57%, 62%, and 31% and in the hull by 43%, 25%, and 36%, for 24 t/ha RSB, 72 t/ha RSB, and 72 t/ha WSB, respectively (Fig. 2).

Mercury content in the roots remained relatively constant over the five treatments, and it was correlated weakly with the Hg content in the other tissues ($0.05 < P$). Unlike upland crops, rice root is coated with iron oxides, namely iron plaques, which is regarded as the first barrier for metal(loid)s crossing the root plasma membrane (Emerson et al., 1999). Iron plaques can absorb inorganic Hg and subsequently deposit on the outlayer of root (e.g., epidermis) (Li et al., 2016). Addition of Fe (II) to soil promoted the accumulation of Hg in the root iron plaques, leading to the enhancement of Hg content in the root tissues (Zhong et al., 2018). Both RSB and WSB treatments resulted in the significantly increase in the concentration of Fe(II) in the pore water of soil (Fig S2,-B,-C), which might strengthen the iron plaques (Zheng et al., 2012) to absorb more Hg on the root surface. Although the leaf Hg content decreased with the biochar treatment, no clear relationship was observed between the leaf and grain Hg contents. This result is likely due to the fact that some of the Hg in the leaf originated from the atmosphere rather than from the soil.

We performed a Pearson correlation analysis to evaluate the Hg content within the different tissues of rice. Notably, positive correlations were observed among Hg contents in the stalk, polished rice, hull, and bran (Table 3). Moreover, a principal component analysis (PCA) was performed (Table S2); one principal component (PC), which could explain over 50% of the data variance, had a high loading for the stalk, polished rice, hull, and bran Hg parameters. Our explanation for these results was that the Hg in these rice tissues might have a common origin. Further, we found that Hg in the pore water was positively correlated with that in the polished rice, hull, bran, and stalk, respectively (Fig. 3), which indicates that a significant portion of the Hg in these rice tissues originated from the pore water. Therefore, the reduction in Hg accumulation observed for the grains grown in biochar-treated soils could be related to the decrease in Hg mobility in the pore water.

Table 3
Pearson linear coefficients (r) matrix for Hg content in the root, stalk, hull, leaf, polished rice and hull ($n = 15$).

	Stalk	Leaf	Polished rice	Bran	Hull
Root	ns	ns	ns	ns	ns
Stalk		ns	0.54^{*}	0.50^{*}	0.75^{**}
Leaf			ns	ns	ns
Polished rice				0.89^{**}	0.51^{*}
Bran					0.53^{*}

*, **, significant levels at $P < 0.05$ and $P < 0.01$ respectively.
ns: not significant.

3.4. The mechanism of Hg reduction in the pore water by the biochars

Biochars remove Hg from solution mainly through their functional groups by forming Hg-S coordination in the presence of high sulfur content, or forming Hg-O/Cl coordination in the presence of low sulfur content (Liu et al., 2016). Unlike solution, sediment or soil is more complex in matrix, the addition of biochar to this matrix may lead to Hg removal through the pathway that differs from the solution. Liu et al. (2017) found that addition of biochar resulted in the decrease of Hg concentration in the pore water of sediment, and Hg was mainly coprecipitated with sulfur, and iron in the biochar because the anaerobic environment of sediment was favoured to produce sulfide to form Hg sulfides. In our study, both RSB and WSB were able to decrease Hg mobility in the pore water of paddy soil, and contained high contents of sulfur of 0.7–1%. We proposed that the elevated sulfur in the biochar might promote the immobilization of Hg through forming Hg-S coordination, as reported by Liu et al. (2016). Further, the RSB was more efficient at reducing Hg mobility in the pore water than the WSB, which we attributed to the former had higher total sulfur content, and its treated paddy soil showed higher sulfate concentration relative to the WSB treatments (Fig. 1-B). The presence of more sulfur might enhance the production of sulfide to precipitate with Hg. The hypothesis is supported by our observation of negative correlation between Hg and sulfate in the pore water (Table 2). Also, our results showed that the presence of more DOC by biochar addition to soil might enhance the Hg mobility in the pore water, as demonstrated by a positive correlation between Hg and DOC in the pore water (Table 2). It seems that the use of biochar to reduce the Hg mobility in the paddy field was a consequence of a joint effect of sulfur and DOC. For instance, RSB treatments, which resulted in a greater extent of increase in sulfate concentration (20–28%) and a slightly increase in DOC concentration (3–21%) (Table S3), showed a greater extent decrease of Hg concentration in the pore water compared to the WSB treatments. The above results also indicate that the production of biochar with high sulfur content and stable organic carbon may enhance the Hg immobilization. The modification of biochar with sulfur-compounds to increase the sulfur level (O'Connor et al., 2018; Liu et al., 2018), and the pyrolysis of plant-based feedstocks at high temperature to increase the stability of carbon in biochar (Singh et al., 2012; Spokas, 2010) might be viable opinions to improve the performance of biochar in Hg immobilization. Further studies should focus on the biogeochemical behaviour of sulfur and organic carbon of biochars after their addition to soils, and their interactions with soil constituents.

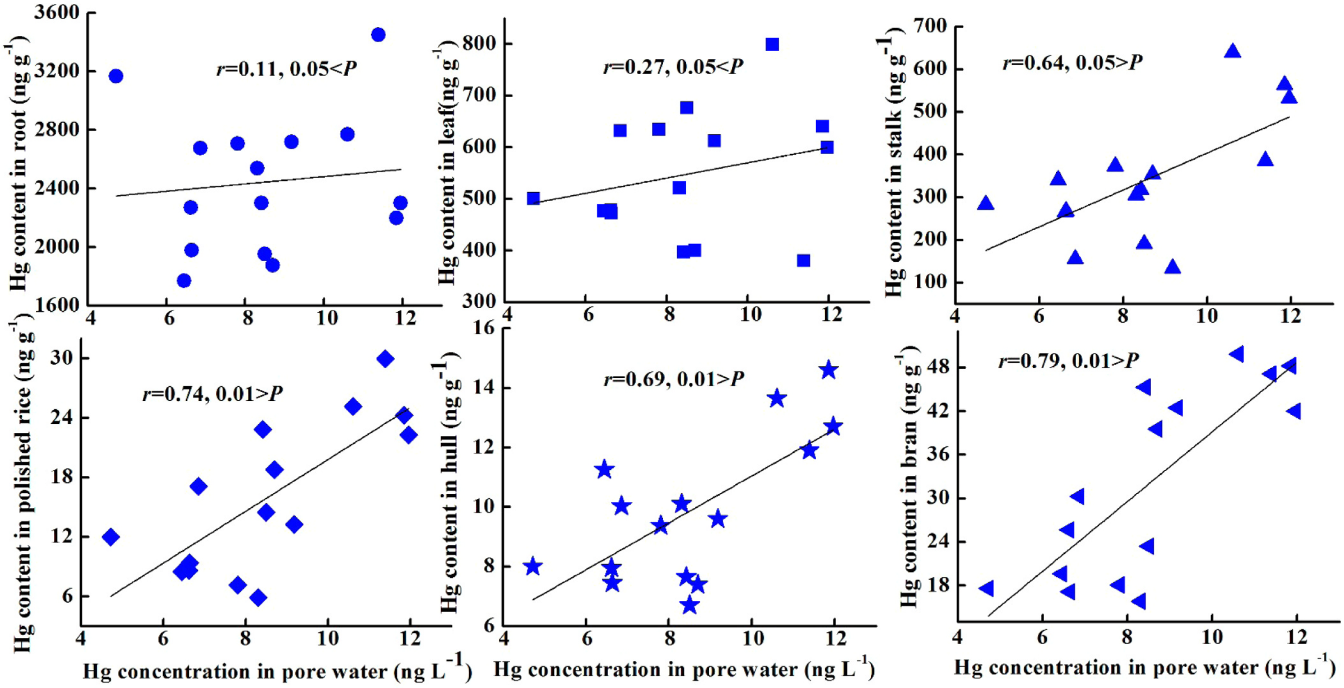

Fig. 3. The relationship between the total Hg concentration in the pore water (the average concentration of Hg in the pore water over the sampling period) and the Hg contents in the different rice plant tissues (root, stalk, leaf, polished grain, bran, and hull) (n = 15).

4. Conclusions

Application of both the WSB and RSB to soils increased the biomass of the aboveground tissues of rice plants, and decreased the Hg levels of the pore water, particularly in RSB treatments. The primary mechanism of this Hg reduction might be related to the formation of Hg sulfides due to the biochar amendment to soils. RSB was more efficient than WSB at reducing Hg accumulation in the stalk, polished rice, bran, and hull. Moreover, rice grown in RSB-treated soil had a Hg level in its grain below the safe Hg level ($< 20\,\text{ng}\,\text{g}^{-1}$) recommended by the Chinese government. Application of biochar (especially RSB) reduced the Hg levels in the rice grains, likely suggesting a potential mechanism of adding biochar to soil to reduce the health risks associated with rice consumption. These results demonstrate that biochar treatment may be an sustainable approach to the management of Hg-polluted soils.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.ecoenv.2018.11.111.

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