Selenium-amended biochar mitigates inorganic mercury and methylmercury accumulation in rice (*Oryza sativa* L.)☆

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ARTICLE INFO

ABSTRACT

Rice, as a dominant crop in China and Asia, can be a major route of methylmercury (MeHg) exposure for humans in inland China, especially in those living in mercury (Hg) polluted areas. Soil is the most prominent MeHg accumulation source for rice grains. The development of management practices to reduce MeHg in rice grains is crucial. This study explored the mitigation effect of biochar (BC) and sodium selenite-amended biochar (BC + Se) on MeHg production in paddy soil and accumulation in rice. Mercury-contaminated soil was treated with 1% and 5% of both BC and BC + Se. Soil MeHg concentration slightly increased under 1% BC/BC + Se compared to control soil but decreased at the rate of 5%. Moreover, soil phytoavailable MeHg (P–MeHg) diminished as the amount of Se-amended BC increased. BC + Se effectively mitigated MeHg accumulation in rice grains. The highest average contents of MeHg and inorganic Hg (IHg) in rice seeds were found in the control samples, followed by the 1%-BC, 5%-BC, 1%-BC + Se, and 5%-BC + Se samples. Under the 5%-BC + Se treatment, rice MeHg levels were reduced significantly (94%) compared to the control, and P–MeHg concentrations in soil were lower than all the other experimental groups throughout the rice-growing season. These results demonstrate the effectiveness of BC + Se in reducing MeHg and IHg accumulation in rice and could be employed for remediation of Hg polluted paddies.

1. Introduction

Mercury (Hg) has received significant attention as a global pollutant because of its methylation to the highly toxic species, methylmercury (MeHg) (Beckers and Rinklebe, 2017; Stein et al., 1996). MeHg exhibits both bioaccumulation and biomagnification in the food chain, posing potential health risks to humans (WHO, 1990). The bioaccumulation of MeHg in rice was confirmed in recent years (Meng et al., 2010; Qiu et al., 2008; Zhang et al., 2010b). Consequently, rice (*Oryza sativa* L.), a dominant global crop, in addition to fish consumption, can also be a major route of human MeHg exposure in inland China, especially in Hg-polluted areas (Zhao et al., 2020; Feng and Qiu, 2008; Zhang et al., 2010a). MeHg-contaminated rice is not only a problem in China but also in other countries such as Pakistan (Aslam et al., 2020), India (Lenka et al., 1992), USA (Windham-Myers et al., 2014), and Indonesia (Krisnayanti et al., 2012), indicating that it is a global issue (Zhao et al., 2020).

Previous studies have confirmed that soil is the predominant source of MeHg for rice plants (Liu et al., 2021; Meng et al., 2010, 2011). Soil contamination degrades food safety and poses health risks. Paddy fields are typical ephemeral wetlands; their anoxic conditions support the methylation of inorganic Hg (IHg) to toxic MeHg (Meng et al., 2011; Zhao et al., 2016a, 2016b), resulting in MeHg accumulation in rice seeds. Thus, the development of management practices to reduce MeHg production in paddy soil and accumulation in rice grains is a critical issue. Several techniques have been used to treat Hg-contaminated farmlands, such as soil washing, desorption, reducing Hg mobility (e. g. water management, sulphate and/or selenium addition, and

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☆ This paper has been recommended for acceptance by Jörg Rinklebe.
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nanoactivated carbon addition), and phytoremediation (Li et al., 2015; Qiu et al., 2014; Wang et al., 2020a, 2019; Wang et al., 2016a, 2016c; Peng et al., 2012). At 350 °C, more than 90% of the Hg in contaminated soil can be removed (Qiu et al., 2014). However, the cost of this method and the effect of heat treatment on the soil properties must be carefully managed. Aerobic rice cultivation reduces Hg accumulation in rice seeds (Peng et al., 2012). Nonetheless, rice growth and yield can be compromised in aerobic conditions because flooded rice fields ensure higher nitrogen and phosphorus fixation levels while reducing competition from weeds and soil-borne pathogens (Li et al., 2015). Hence, developing alternatives to mitigate MeHg accumulation in rice while providing optimal growth conditions is highly desired.

As a cheap, multi-benefit, porous, and carbon-rich material, biochar (BC) has been increasingly applied in agriculture and environmental remediation applications. Because of its relatively high specific surface area, it has been tested in previous studies and has been shown to remediate heavy metal-contaminated soils (Shu et al., 2016b; Zhang et al., 2018). Increasing attention has been paid to the use of BC to reduce the bioavailability of pollutants, reduce the absorption of contaminants by plants, and enhance the activity of microorganisms to promote the degradation of organic pollutants in soil (Beesley et al., 2010) used sugarcane straw-derived BC to remediate soil pollution with multiple heavy metals. BC amendment has also been found to lower the accumulation of MeHg in plants (e.g., mustard and rice; Shu et al., 2016a, 2016b; Xing et al., 2020; Zhang et al., 2018).

In some studies, the maximum application of BC was 10–20% in order to achieve acceptable heavy metal stabilization efficiency (Wang et al., 2020b). However, for agricultural operations, higher application rates increase the risk of soil alkalinity and reduce crop biomass (Khan et al., 2015). More importantly, the unbalanced nutrient content of BC could reduce seed germination (Beesley et al., 2013). Furthermore, heavy application of BC increases soil Hg methylation (Shu et al., 2016a, 2016b), which impacts the watershed and increases the risk of MeHg pollution. To improve the stabilization efficiency of BC on heavy metals, it is not possible to simply increase the amount of BC applied. Modification of BC is considered an effective approach to address this problem (Li et al., 2017). Hydroxides, organic solvents, iron compounds, and acids may all be used to modify BC (Lu et al., 2018).

Selenium (Se) is an essential trace element that antagonizes the toxicity of Hg and many heavy metals such as Cr, and Cd (Wang et al., 2016a). The amount of bioavailable IHg and MeHg in the root zone is controlled by forming covalent Hg–Se insoluble bonds in the rhizospheres and/or roots (Zhang et al., 2012). Further, several pot experiments have indicated that Se could be complexed with IHg, consequently reducing methylation potential of IHg in soil and accumulation in rice (Wang et al., 2014, 2016a; 2016b). Several researchers have discussed BC and Se amendment for the treatment of Hg pollution in soil and MeHg accumulation in rice; however, no published studies have yet evaluated the effects of the combined application of BC and Se on the reduction of MeHg production in paddy soils and accumulation in rice. The current study was designed to assess the effectiveness of Se-amended BC on reducing (1) MeHg production and its bioavailability in paddy soil and (2) MeHg uptake and accumulation in rice plants.

2. Materials and methods

2.1. Experimental design

The soil for the pot experiment was collected from a Hg-polluted paddy (1–10 cm) situated at an artisanal Hg mining site (Gouxi) in the Wanshan Hg mining area, Guizhou province, China. Before use, the soil was ground and sieved through a 10 mesh. Hg contamination of the ambient air, stream water, soil, and organisms is a severe consequence of artisanal Hg smelting activities (Li et al., 2008). Atmospheric Hg deposition is the predominant Hg source in local paddy soil (Zhao et al., 2016a, 2016b). More importantly, newly deposited Hg is more readily

methylated than pre-existing Hg in paddy soils (Meng et al., 2011). As a result, MeHg levels in paddy soil and rice seeds from artisanal Hg mining areas were found to be 1–2 orders of magnitude higher than those in a large-scale abandoned Hg mining area (Meng et al., 2010; Zhao et al., 2016a, 2016b).

Rice hull BC was obtained from the Engineering Research Center of Biochar of Zhejiang Province, China. A muffle furnace produced the rice straw BC (pyrolysed at 550 °C for 2 h) under a limited oxygen supply. The rice straw BC was ground and sieved through a 50 mesh (300 μm) to prepare the modified BC. A certain amount of sodium selenite (Na$_2$SeO$_3$, 1401.7 g) was dissolved in water and then mixed thoroughly with the BC powder (the mass ratio of Se/BC was kept at 1:1000) in a 2 L beaker. The mixture was evenly shaken for 12 h in an oscillating chamber and dried to a constant weight in a vacuum dryer to obtain sodium selenite-modified biochar (BC + Se). Assuming that all of the sodium selenite in the mixture was absorbed by biochar, the nominal Se concentration in selenium-amended biochar is 1000 mg kg^{-1}.

In this study, 5 treatments (2 BC treatments, 2 BC + Se treatments, and the control) and 15 pots (3 replicates for each treatment) were evaluated (Table S1). Different amounts of BC (1% or 5%) and BC + Se (1% or 5%) were incorporated into the soil. Relatively low BC application rates (1–5%) were utilized in the current study to investigate the potential remediation of Hg-contaminated farmland at a low cost; nonetheless, the application rates chosen were within the typical application rate range (1–10%; Trakal et al., 2011). The control consisted of soil without the addition of BC. The amended soils were thoroughly mixed before being placed in 8 L polypropylene pots (3 kg soil per pot). A rice cultivar (hybrid rice) widely grown throughout Guizhou province was selected for the present experiment. On June 9, 2018, hybrid rice seedlings (30 days old, cultivated in low-Hg soil) were transplanted into the experimental pots (2 seedlings per pot, 3 replicates per treatment); the rice was harvested on October 9, 2018. The water level was maintained at 2–4 cm for each pot to induce anoxic soil conditions for optimal rice growth. Anaerobic soil environment was provided in pots, similar to actual field conditions. At ambient temperature, the pots were placed in a greenhouse.

2.2. Sample collection and preparation

Four sampling campaigns were conducted in this study during the rice-growing season. The first sampling was carried out 20 days after rice seedling transplantation, and the remaining sampling occurred on day 60, 80, and 120. For each sampling campaign, soil samples from the rhizosphere were collected from the experimental pots. The rhizosphere soils were collected by hand while wearing disposable polyethylene gloves, packed in double bags, stored in a cold container, and transported to the laboratory within 24 h. In the laboratory, they were freeze-dried in a refrigerator (FD-3-85D-MP, FTS, USA). The freeze-dried soil samples were homogenized through 200 mesh with a mortar before analysis. The concentrations of total Hg (THg) and MeHg, and the phytoavailability of MeHg in the soil samples were determined.

In contrast, rice samples were collected into polyethylene bags on day 120 during the ripening period. The rice samples were air-dried in the laboratory. The rice seeds (brown rice) were separated from their hulls in the laboratory using a huller (JLGJ-45) and ground through 150 mesh (IKA-A11 basic, IKA). Precautions were taken during sample preparation to prevent cross-contamination. Following processing of each sample, the grinder was thoroughly cleaned. The powdered samples were kept in Ziplock bags and stored in a refrigerator at 4 °C in the dark before THg and MeHg analysis.

2.3. Analytical methods

All reagents were of analytical grade and obtained from Shanghai Chemicals Company (Shanghai, China). THg concentrations in rice and soil samples were determined using a direct mercury analyzer (DMA-80,

Milestone, Italy) at the Institute of Geochemistry, Chinese Academy of Sciences (IGCAS). For MeHg analysis, a 0.3–0.5 g rice grain sample was digested using the KOH-methanol/solvent extraction method. Then, the digestion was acidified using concentrated HCl. A 0.3–0.4 g of soil sample was prepared using the $CuSO_4$-methanol/solvent extraction technique (Liang et al., 1996). MeHg was extracted from each sample using methylene chloride, back-extracted from the solvent phase into water, and then later ethylated by addition of 0.1 mL 1% (v/v) NaB $(C_2H_5)_4$ (sodium tetraethylborate). By purging with N_2 onto a Tenax trap, the ethyl analogue of MeHg, methylethylmercury $(CH_3HgCH_2CH_3)$, was separated from the solution. The trapped $CH_3HgCH_2CH_3$ was thermally desorbed, separated from other Hg species using an isothermal gas chromatography column (GC), and decomposed to Hg^0 in a pyrolytic decomposition column (800 °C) before being detected by cold vapor atomic fluorescence spectrometry (CVAFS, Brooks Rand Model III, Brooks Rand Labs, Seattle, WA, USA) at IGCAS following the USEPA method (USEPA, 2001, 2017). Phytoavailable MeHg (P–MeHg) in the soil samples was determined using the ammonium thiosulphate extraction method (Zhu et al., 2015). Briefly, the soil sample was mixed with ammonium thiosulphate solution (2 g L^{-1}) and prepared with deoxygenated water at a ratio of 2.5 mL g^{-1} in polypropylene centrifuge tubes within a glove box filled with nitrogen gas. The tubes were capped and shaken for 12 h in an incubating shaker (25 °C). After this, the mixtures were centrifuged at 2900 RPM for 15 min. Finally, the MeHg concentrations in the supernatant solutions were determined.

Soil pH was measured using a pH electrode (PD-188 501, SANXIN, China) with a solid: water ratio of 1:2.5. The potassium dichromate volumetric method, combined with the water heating technique, was used to determine the organic matter content of soil samples (Lu, 2000; USDA, 2014). The IHg concentrations in rice grains were calculated as the difference between the concentrations of THg and MeHg in samples (Meng et al., 2010).

2.4. Quality control, calculation and statistical analysis

Quality control for the THg and MeHg analysis was ensured by conducting triplicate analysis, method blanks, and certified reference materials. The detection limits of THg and MeHg in the rice and soil samples were 0.01 µg kg^{-1} and 0.003 µg kg^{-1} (3σ), respectively. For analysis of triplicate samples, relative standard deviation (RSD%) was less than 7.4% for THg and 9.3% for MeHg for both soil and rice plant samples. Various certified reference materials, including Citrus leaf (GBW10020, National Research Centre for Certified Reference Materials) and sediment (GBW08308, National Research Centre for Certified Reference Materials), Lobster Hepatopancreas (TORT-3, National Research Council Canada), Montana II soil (SRM-2711a, National Institute of Standards and Technology) and Sandy Loam 3 (CRM021-100, Resource Technology Corporation) were used for quality control in this study, and the corresponding analytical results are shown in Table 1.

The reduction rates of MeHg and IHg in rice grains under the different treatments were calculated using the following Equation:

$$R = \left[Hg_C^X\right] - \left[Hg_T^X\right] / \left[Hg_C^X\right]$$

Where R is the reduction rate; X is Hg species IHg/MeHg; C is Hg concentration in control; and T is Hg concentration in treatments.

Statistical analysis of the sampling data was performed using the statistical package SPSS 21 software (SPSS China Ltd., Shanghai, China). Measurements of THg and MeHg are usually described as mean ± standard deviation (SD). Correlations between covariant sets of data were subjected to regression analysis. For linear regression fits, correlation coefficients (r) and significance probabilities (p) were computed. One-way ANOVA with the LSD test was used to compare the significance of different treatment groups at $p < 0.05$.

Table 1
List of certified reference materials used in the present study and corresponding analytical result.

Producer	CRM	n	Hg speciation	Obtained value	Certified value
NRC CRM	GBW10020	5	THg (µg kg^{-1})	158 ± 0.83	150 ± 20
NRC CRM	GBW08308	5	MeHg (µg kg^{-1})	3.9 ± 0.21	5.2 ± 0.7
NRCC	TORT-3	10	MeHg (µg kg^{-1})	108 ± 8.6	137 ± 12
RTC	CRM021-100	6	THg (mg kg^{-1})	4.5 ± 0.24	4.7
NIST	SRM-2711a	5	THg (mg kg^{-1})	6.6 ± 0.33	7.42 ± 0.18

CRM: certified reference material. NRC CRM: National Research Centre for Certified Reference Materials. NRCC: National Research Council Canada. RTC: Resource Technology Corporation. NIST: National Institute of Standards and Technology.

3. Results and discussion

3.1. Organic matter contents and pH in soil

Soil pH and organic matter (OM%) levels during the rice-growing season are shown in Table S2. The OM% average (range) values in the control, 1%-BC, 5%-BC, 1%-BC + Se, and 5%-BC + Se soil were 4.7 ± 0.56 (3.0–6.2), 5.4 ± 0.64 (4.8–6.1), 5.7 ± 0.93 (5.0–7.1), 4.9 ± 1.0 (3.8–6.0), and 6.0 ± 1.2 (4.8–7.2), respectively. The OM% increased with increasing amounts of BC and BC + Se. However, OM% slightly decreased till harvesting from day 60 (Fig. S1). The pH values in the soil samples were similar among the treatments, ranging from 7.4 to 7.8. It could be owing to the comparatively low rates of BC (1% or 5%) applied in this study. Another probability is that root exudation in rice paddy may reduce soil pH (Rothenberg and Feng, 2012), neutralizing the effects of BC amendment on soil pH.

3.2. Total mercury and methylmercury in soil

During the rice-growing season, the average concentrations of THg in the control and treatment soils were 14 ± 1.3 mg kg^{-1}, 14 ± 2.8 mg kg^{-1} (1%-BC), 15 ± 1.3 mg kg^{-1} (5%-BC), 16 ± 2.9 mg kg^{-1} (1%-BC + Se), and 14 ± 1.5 mg kg^{-1} (5%-BC + Se), respectively. These consistently exceed the domestic environmental quality guideline for agricultural soil (pH > 7.5, 1.0 mg kg^{-1}, CNSA, 2018). There were no discernible differences in the average concentration of THg among the control and treatment soils (1%-BC, 5%-BC, 1%-BC + Se, and 5%-BC + Se), indicating that the THg content was unaffected by the soil treatments (BC and BC + Se).

In contrast, changes to soil MeHg were evident among the treatment pots, with the MeHg levels differing between the treatments (Table S2). The concentrations (average value during the rice-growing period) of MeHg in soil samples from the treatment and control pots are depicted in Fig. 1a. In general, the BC (1% and 5%)-amended soil had significantly higher MeHg concentrations ($p < 0.05$) than the control soil and the BC + Se soil (1% and 5%) during the rice growth period (Fig. 1a) ($p < 0.05$). Thus, BC amendment resulted in higher MeHg concentrations, especially with the 1%-BC treatment; the highest concentration was 8.9 ± 1.1 µg kg^{-1}, indicating that BC amendment can increase net MeHg production in soil.

The increase in soil MeHg levels observed under BC amendment partially contradicts previous studies that have reported that BC can decrease MeHg production in sediments by inhibiting Hg bioavailability (Bussan et al., 2016). In the current study, increasing BC and BC + Se amendment from 1% to 5% led to a decrease in MeHg. The recorded MeHg in the treated soil (BC and BC + Se) was 1.2–2.7 orders of magnitude higher than MeHg in the control soil. This result is consistent
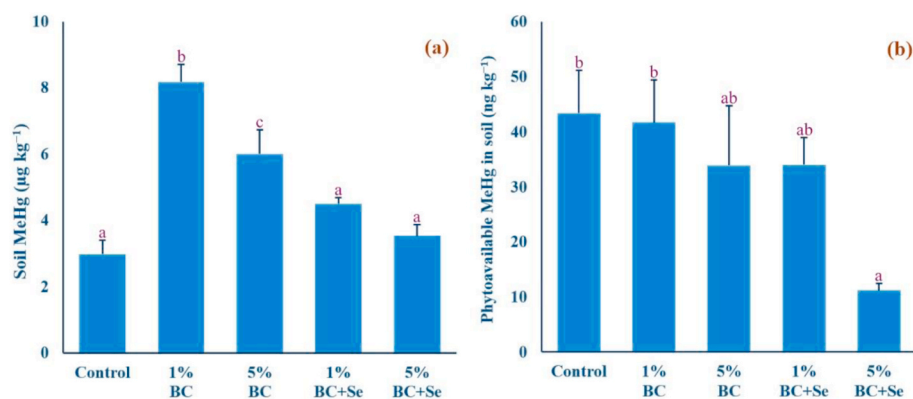
Fig. 1. Average MeHg (A) and phytoavailable MeHg (B) concentrations in the soil during the rice growing season of four period at different pot experiments treatments. Data are presented as Mean ± standard deviation (SD), n = 3. Different letters above the bars (a–c) denote significant differences among treatments for a certain time point (one-way ANOVA, $p < 0.05$).

with that of Shu et al. (2016b), who found that amendment with straw BC increased MeHg concentrations in paddy soils by 36–303%. The increase in soil MeHg may be related to a change in microbial activity and the production of MeHg by microorganisms (Shu et al., 2016b). A subset of sulphate-reducing bacteria (SRB) greatly facilitates Hg methylation (Gilmour et al., 1992). Moreover, a recent study has also confirmed that SRB plays a significant role in MeHg production in soil, while methanogen plays a minor role (Wang et al., 2016a). Sulphate levels significantly affect SRB activities, affecting MeHg production in soils (Gilmour et al., 1992). A previous study found that high soluble sulphate levels in BC and in BC-amended soil enhanced the activity of SRB activity and facilitated the production of microbial MeHg from SRB (Shu et al., 2016b). In other words, the increase in SRB activity caused by BC may be one of the reasons for the elevated soil MeHg. Therefore, we hypothesize that the increased MeHg production in the soils amended with BC may be due to the high sulphate content of BC. However, on the other hand, due to its relatively high specific surface area and high affinity to Hg, excess BC could reduce the bioavailability of Hg, and then inhibit the conversion of inorganic Hg to MeHg. Accordingly, MeHg levels with the 5%-BC treatment were significantly lower than those with the 1%-BC during the rice-growing season ($p < 0.05$).

As shown in Fig. 1a, MeHg concentrations in soil samples from the 1%-BC + Se and 5%-BC + Se treatments were significantly lower than those in the 1%-BC and 5%-BC treatments, which were slightly higher than the control soil but not significantly different (one-way ANOVA, $p < 0.05$). This suggests that Se in soil plays a key role in net MeHg production. In the preceding paragraph, we argued that SRB plays a key role in MeHg production; an inhibitory effect of Se addition on MeHg production by SRB was found in bacterial culture experiments (Truong et al., 2013). Se can also directly affect microorganisms that regulate Hg methylation, with Se addition promoting demethylation and evaporation of MeHg (Dang et al., 2019). On the other hand, the relatively lower MeHg concentrations in the 1%-BC + Se and 5%-BC + Se treatment soils could primarily be attributed to the formation of Hg–Se complexes, and thus, decreased microbial Hg methylation (Wang et al., 2016c), which reduces the impact of BC amendment on soil MeHg production.

3.3. Se-amended BC inhibits the phytoavailability of MeHg in soil

A previous study reported that extractable MeHg by $(NH_4)_2S_2O_3$ is indicative of phytoavailable MeHg (P–MeHg) in paddy soil (Zhu et al., 2015). Average soil P–MeHg levels are shown in Fig. 1b and were calculated as the MeHg level in supernatant solution after $(NH_4)_2S_2O_3$ extraction (ng MeHg)/Mass of the soil used for extraction (kg soil). Our results showed that phytoavailable MeHg levels (ng kg^{-1}) in the treatment and control soils declined as follows: control (43 ± 6.7)>1%-BC (42 ± 3.4)>1%-BC + Se (34 ± 5.1)>5%-BC (33.9 ± 4.)>5%-BC + Se

(11 ± 3.7). Generally, the recorded P–MeHg values in the soil treated with BC and BC + were lower than the control soil. P–MeHg levels in Se-amended BC (1%-BC + Se and 5%-BC + Se) were significantly lower than in BC-treated soil (1%-BC and 5%-BC) ($p < 0.05$). Furthermore, the lowest concentrations of P–MeHg in soil were observed in the 5%-BC + Se treatment (Table S2 and Fig. 1b), compared with the control and 1%-BC treatment ($p < 0.05$). Soil MeHg concentrations increased under the BC and BC + Se treatments, while phytoavailable MeHg levels in Se-amended BC soil decreased significantly.

Recent studies have shown that BC can control MeHg mobility or bioavailability in soil and sediments (Bussan et al., 2016; Shu et al., 2016b). The MeHg immobilization by BC amendment is mainly due to the large surface area of BC (Ryu et al., 2015); this can make MeHg sorption easier with BC (Gomez-Eyles et al., 2013). Furthermore, several studies have found that BC contains a high concentration of organic sulfur groups (Cheah et al., 2014). Given the high affinity of MeHg to organosulfur groups (Ravichandran, 2004), it is difficult for the MeHg that is adsorbed by BC to be desorbed from soil particles and absorbed by rice roots. On the other hand, the main effect of Se on inhibition of soil MeHg production is a reduction in Hg bioavailability by the formation of dissolved Hg–Se complexes. Se-adsorbed onto BC can be converted to selenide, elemental Se, and/or organic Se in soils (Wang et al., 2016b). Due to Hg's high affinity for Se (George et al., 2011), very low solubility Hg–Se complexes could form in soils, making them less bioavailable for microbial methylation (e.g., SRB, Li et al., 2015; Wang et al., 2016b). Under the combined action of Se and BC, Se firstly reduced MeHg production in soil; then, BC adsorbed MeHg. As a result, soil phytoavailable MeHg levels in the BC + Se treatments were significantly lower than in the control and BC treatments.

3.4. Se-amended BC reduces Hg species accumulation in rice seeds

The average concentrations of THg, MeHg, and IHg in rice samples (brown rice) are summarized in Table S3. The mean THg concentrations in all rice samples, except the 5%-BC + Se treatment, exceeded the allowable limit of 20 µg kg^{-1} for cereal grains, as recommended by the Chinese National Standard Agency (CNSA, 2017). The maximum average THg concentration in rice seeds was found in the control samples (91 ± 10 µg kg^{-1}), and the lowest was in the 5%-BC + Se treatment (4.7 ± 0.87 µg kg^{-1}).

Fig. 2 shows the MeHg and IHg concentrations in rice grains; significant differences were observed among the different treatments ($p < 0.01$). Rice seeds in the control samples accumulated the highest concentrations of MeHg (36 ± 5.6 µg kg^{-1}), followed by the 1%-BC (31 ± 1.8 µg kg^{-1}), 5%-BC (28 ± 1.1 µg kg^{-1}), 1%-BC + Se (25 ± 2.8 µg kg^{-1}), and 5%-BC + Se (2.1 ± 0.4 µg kg^{-1}) treatments (Table S3). Compared to the control samples, the concentrations of MeHg in rice submitted to BC
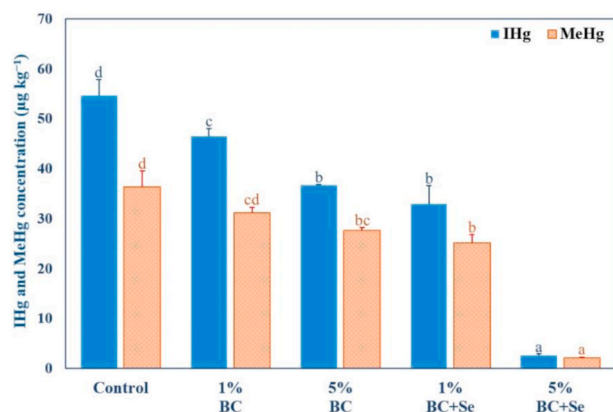
Fig. 2. IHg and MeHg concentrations in rice grains at different pot experiments treatments, letters on the top of bars (a–d) indicate significant differences among treatments (one-way ANOVA, $p < 0.05$). Data are presented as Mean $\pm$ SD, n = 3.

Fig. 3. Reduction rates of MeHg and IHg in rice grains at different treatments of pot experiments. The bars represent the standard deviation (SD). Different alphabets depict significant differences among treatments (one-way ANOVA, $p < 0.05$).

treatment were significantly decreased. Despite the increased MeHg concentrations in soil under BC amendment (Fig. 1a), BC treatment reduced the amount of bioavailable MeHg in the soil (Fig. 1b), leading to a decrease in the amount of MeHg in rice grains. Fig. 2 shows that the MeHg concentration levels in the BC + Se treatments were significantly lower than the control and BC treatments, especially for the 5%-BC + Se treatment. There are two probable reasons for this; MeHg in soil decreased might be due to the impact of BC + Se on net Hg methylation potential in soil (Fig. 1a). On the other hand, the addition of BC + Se significantly decreased the bioavailability of MeHg in the soil (Fig. 1b). Therefore, under the combined influence of these two effects, the concentrations of MeHg in rice grains in the BC + Se treatments were lower than that of the BC treatment and the control. The lowest concentrations of MeHg and phytoavailable MeHg in soil were found with the addition of 5%-BC + Se, which resulted in the lowest concentration of MeHg in rice seeds (Fig. 2).

The concentrations of IHg in rice also exhibited the same trend as that of MeHg (Fig. 2). As shown, the control rice samples (55 ± 5.4 µg kg^{-1}) had the highest IHg contents, while the lowest IHg content was found in the 5%-BC + Se treatment samples (2.6 ± 0.5 µg kg^{-1}) (Table S3). According to the available data, the sources and mechanisms of IHg and MeHg uptake into rice plant tissues could be different (Meng et al., 2010, 2011; 2012). It has been observed that soil is a potential source of MeHg for rice plant tissues (Meng et al., 2010, 2011). However, Hg in paddy soil is the main source of IHg in the roots, while the husk, stalk, and leaves of rice plants can accumulate Hg from the air (Meng et al., 2010, 2012). In this study, we found that paddy soil was also an important source of IHg in rice plants, which was largely underestimated previously (Aslam et al., 2022; Liu et al., 2021). Based on the soil THg and MeHg data, it can be inferred that the addition of BC and BC + Se to paddy soil reduced the bioavailability of both IHg and MeHg in the soil. As a result, the concentrations of IHg in rice under the BC and BC + Se treatments were decreased compared with the control; the effects of the BC + Se treatment were more obvious.

The findings of the current study demonstrate that BC + Se and BC amendments can reduce the accumulation of MeHg and IHg in rice, with the former producing more prominent effects. The decreased MeHg and IHg accumulation rates in rice grains under the different treatments are shown in Fig. 3. Compared to the control, the reduction rates of IHg under the 1%-BC (15%) and 5%-BC (33%) treatments were lower than that of the 1%-BC + Se (40%) and 5%-BC + Se (95%) treatments. More importantly, the reduction rates of the MeHg under the 1%-BC + Se (31%) and 5%-BC + Se (94%) treatments were higher than those of the 1%-BC (14%) and 5%-BC (24%) treatments. The reduction rates of MeHg in rice under the 5%-BC + Se treatment were higher than those
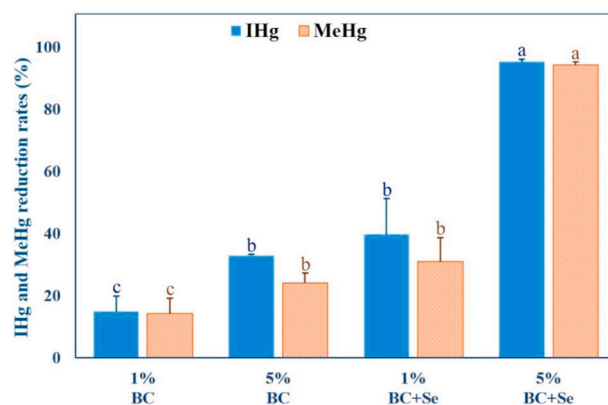
reported in previous studies that used BC amendment to reduce MeHg (Shu et al., 2016b; Xing et al., 2020). BC + Se was more effective than BC in reducing the accumulation of MeHg in rice. The tolerable intake of MeHg recommended by the FAO/WHO (FAO/WHO, 2011; WHO, 2007) is 1.6 µg kg^{-1} body weight per week and the USEPA reference dose (RfD) for MeHg is 0.1 µg kg^{-1} body weight per day (USEPA, 1997). Suppose that the average daily intake of rice is 250 g and the average body weight is 60 kg, the daily MeHg intake for the control rice would be 0.15 µg kg^{-1}, and for the 1%-BC 0.13 µg kg^{-1}, 5%-BC 0.11 µg kg^{-1}, and 1%-BC + Se 0.11 µg kg^{-1}, which all exceed the USEPA recommended limits; the only exception is the 5%- BC + Se treatment (0.009 µg kg^{-1}).

Previous studies have shown that MeHg immobilization under BC amendment may be due to the large relative surface area of BC, which provides sufficient binding sites for metals (Gomez-Eyles et al., 2013). Accordingly, the decline in phytoavailable MeHg could be due to MeHg immobilization in BC-amended soils. Further, BC consists of high levels of organosulfur groups with an affinity to bind to Hg (Ravichandran, 2004). It is speculated that MeHg and IHg adsorbed by BC could hardly desorb from soil particles. However, the mechanisms of the association between MeHg and BC, as well as that underlying the low phytoavailability of MeHg are still not fully understood.

Further developments are needed in this regard. Previous studies have shown that Se can protect against Hg toxicity (Bjerregaard and Christensen, 2012). The high affinity of Se towards Hg leads to the formation of Hg–Se complexes. Suppression of MeHg translocation within plants and/or possible complexation of IHg and Se in soil are hypothesized to alleviate MeHg accumulation in rice following Se addition, i.e., MeHg–Se antagonism (Wang et al., 2014; Zhang et al., 2012). Se could form Hg–Se complexes with higher molecular weights in rice root but may not be transferred to the aboveground part of the rice plant. Wang et al. (2014) found that more than 80% of the total Hg was found in the root biomass in four different rice varieties. Several studies have shown that selenite can protect rice from Hg toxicity and inhibit the accumulation of Hg in rice tissues (Wang et al., 2014). The decrease in MeHg and IHg in rice treated with BC was only due to the adsorption and fixation of bioavailable Hg by BC. Though, under the BC + Se treatment, BC and Se played dual roles, so the reduction effect was more apparent than with BC alone. BC + Se could effectively reduce the risk of human exposure to MeHg by mitigating the accumulation of MeHg in rice. Therefore, BC + Se amendment may be a promising approach to mitigate MeHg accumulation in rice.

4. Conclusions and implications

Conclusively, soil MeHg levels under BC + Se and BC amendments were 1.2–2.7 orders of magnitude higher than those of the control soil.

On the other hand, MeHg concentrations were significantly reduced in rice grain under the BC + Se and BC amendments. The 5%- BC + Se treatment was the most effective in reducing the bioavailability of MeHg in Hg-contaminated paddy soil. This study observed that reduced MeHg and IHg uptake by rice plants resulted in decreased grain MeHg and IHg levels under BC + Se amendment. The control rice seeds accumulated the highest concentrations of MeHg and IHg, followed by the 1%-BC, 5%-BC, 1%- BC + Se, and 5%- BC + Se rice seeds. The MeHg and IHg concentrations in rice declined at a rate of 94% and 95% for the 5%- BC + Se treatment compared to the control. BC and Se played a dual role, so the reduction effect was more significant than that of BC alone. Thus, the current study provides an effective way to reduce MeHg accumulation in rice grains. However, further studies are needed to fully understand the underlying mechanisms of BC and Se application in rice paddies under actual field conditions. In addition to the above reasons, BC amendment-enhanced root growth might play an important role in MeHg uptake by rice and its transport in rice plants. The current study demonstrates the effectiveness of BC + Se in reducing MeHg and IHg accumulation in rice and may provide a theoretical basis for remediation of Hg polluted paddy soil. Further large-scale field studies are required to validate the usefulness of BC + Se application in rice paddies.

Author statement

Wenqiang Lv: Formal analysis, Investigation, Visualization, Validation, Writing-Original Draft. **Tianli Zhan**: Formal analysis, Investigation, Visualization, Writing-Original Draft. **Mahmoud A. Abdelhafiz:** Methodology, Writing-Review & Editing. **Xinbin Feng**: Supervision, Funding acquisition, Conceptualization, Writing-Review & Editing. **Bo Meng**: Funding acquisition, Conceptualization, Supervision, Investigation, Writing-Review & Editing. All authors read and approved the final manuscript.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgment

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envpol.2021.118259.

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