Science of the Total Environment 666 (2019) 1126–1133

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

# Biochar facilitated the phytoremediation of cadmium contaminated sediments: Metal behavior, plant toxicity, and microbial activity

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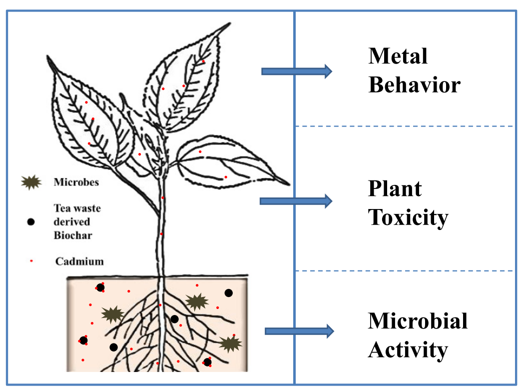
## HIGHLIGHTS

- TB was prepared according to the pyrolysis of tea waste.
- TB changed Cd speciation in river sediments.
- TB increased Cd accumulation and translocation in the plants.
- TB alleviated Cd induced toxicity to plants and microbes.
- TB enhanced the phytoremediation efficiency and reduced the risks of heavy metals.

## GRAPHICAL ABSTRACT




## ARTICLE INFO

*Article history:*
Received 6 January 2019
Received in revised form 13 February 2019
Accepted 13 February 2019
Available online 14 February 2019

Editor: Frederic Coulon

*Keywords:*
Phytoremediation
Cadmium
Plants
Microbes
Sediments

## ABSTRACT

Cadmium (Cd) contamination in river sediments becomes increasingly serious, and phytoremediation has been used to remediate Cd contaminated sediments, but the remediation efficiency needs to be improved. In this study, tea waste derived biochar (TB) was used to facilitate the phytoremediation of Cd contaminated sediments. Results showed that TB at 100, 500 and 1000 mg kg$^{-1}$ increased Cd accumulation and translocation in ramie seedlings by changing Cd speciation in sediments and altering the subcellular distribution of Cd in plant cells. TB at low contents alleviated Cd induced toxicity in ramie seedlings by promoting plant growth and mitigating the oxidative stress. In addition, the activities of urease-, phosphatase-, and catalase-producing microbes in the Cd contaminated sediments were promoted by the application of TB. These findings demonstrated that biochar at low concentrations could improve the phytoremediation efficiency and mitigating Cd-induced toxicity to plants and microbes in Cd contaminated sediments. This study herein provides a novel technological application of waste biomass in controlling and mitigating risks of heavy metals.

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## 1. Introduction

Heavy metals are existed diffusely in the environment. River sediments serve as the major sinks and migration destinations for the heavy metals (Huang et al., 2018a; Xue et al., 2017). Nowadays, heavy metal pollution in the river sediments becomes increasingly serious

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https://doi.org/10.1016/j.scitotenv.2019.02.215
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due to the widely use and arbitrary discharge of heavy metals. Cadmium (Cd), known as a common toxic heavy metal in the river sediments, can be absorbed by aquatic organisms and bioaccumulated through food chain, thus posing a potential threat to human health and the ecosystem (Huang et al., 2018b). Hence, it is imperative to remediate Cd-contaminated river sediments. Recently, the applications of amendments and phytoremediation in the remediation of Cd-contaminated river sediments have made progress (Gong et al., 2017a; Huang et al., 2017a).

Biochar, a carbonaceous product producing from the pyrolysis of biomass under no or oxygen-limited condition, is regard as a promising amendment to remedy environmental pollution due to its specific physicochemical properties, such as porous structure, high surface area, and abundant surface functional groups (Gong et al., 2018b; Lawrinenko et al., 2016; Leng et al., 2019). The application of biochar was reported to immobilize heavy metals in the environment and reduce the biotoxicity of heavy metals to the organisms (Gong et al., 2018b; Zhang et al., 2019). For example, Xu et al. demonstrated that bamboo biochar could immobilized Cd and Pb in the soil and reduced their bioavailability, and they ascribed these results to the adsorption of metals to the surface of biochar by complexation and ion exchange (Xu et al., 2016). However, the immobilization and reduced bioavailability of heavy metals in the environment by biochar is not conducive to the uptake of heavy metals by plants. Inversely, a study conducted by Rees et al. demonstrated that besides immobilizing metals, biochar also could adsorb nutrients, which resulted in plant nutrients deficiency or reduced the competition between nutrients and heavy metal ions, thus enhancing the extraction of heavy metals by hyperaccumulators (Rees et al., 2015). Since Cd and nutrients could compete for adsorption sites on biochar and share the entrance channels and transporters to get into plants, it is unclear how can biochar influence the behavior of Cd in the sediments and plants. In addition, biochar was reported to alleviate the toxic effects of plants under abiotic stress. For example, Farhangi-Abriz demonstrated that biochar protected bean seedlings against salt stress by enhancing the shoot and root dry weight and alleviating the oxidative stress (Farhangi-Abriz and Torabian, 2017). Rizwan et al. summarized the mechanism of biochar-mediated toxicity alleviation in plants (Rizwan et al., 2016). However, to our knowledge, very few studies have investigated the impacts of biochar on heavy metal induced toxicity, especially the oxidative damage, in plants growing in river sediments. Besides, biochar has been found to positively affect the microbial activity in the contaminated environment. For example, Sugarcane bagasse derived biochar enhanced the population of bacteria and actinomycetes, and increased the soil enzyme activities in the Cd, Cu and Pb contaminated soil (Nie et al., 2018). However, little is known about the effects of biochar on the microbial activity in the heavy metal contaminated environment where plants are growing.

Biochar could change the behavior of heavy metals and modify the microbial activity directly in the contaminated sites without plants (Dell'Anno et al., 2003; Jin et al., 2011; Nie et al., 2018). However, with plants cultivation, the effects of biochar were likely to be changed, which might influence the remediation of heavy metal contaminated sites. The interaction among biochar, plants and microbes might alter the heavy metals behavior in the environment and plants. The beneficial roles of biochar on plant growth and on the enhancement of microbial activity were likely to improve the phytoremediation efficiency. To our knowledge, studies on how can biochar influence metals behavior, plant toxicity and microbial activity in the heavy metal contaminated sediments growing with plants are limited. Thus, in this study, the roles of tea waste derived biochar (TB) in the Cd contaminated river sediments cultivating with *Boehmeria nivea* (L.) Gaudich (ramie) seedlings were investigated. Tea waste is a kind of waste biomass that needs to be handled, and recent reports demonstrated that using TB is a promising low cost remedy for pollutants removal from the environment (Rajapaksha et al., 2014). Thus, tea waste was used as the raw material of biochar in this experiment. Ramie seedlings were chosen as the text plants due to their high Cd accumulation capacity and rapid growth rate (Gong et al., 2016). Cd speciation in the sediments, the accumulation and translocation of Cd in ramie seedlings and the subcellular distribution of Cd in plant cells were determined to assess the effects of TB on metal behavior in the Cd contaminated sediments. Plant biomass and the oxidative damage in ramie seedlings were measured to explore the roles of TB on plant toxicity. The microbial activity in this Cd contaminated sediments was analyzed by determining the activities of microbial enzymes. The aims of this work were to investigate the roles of biochar in heavy metal contaminated sediments, and to determine the impacts of biochar on the phytoremediation of heavy metal contaminated sites.

## 2. Materials and methods

### 2.1. Sediments and biochar preparations

Cd contaminated sediments were obtained from the Xiangjiang River in Changsha, China. Biochar was obtained from the pyrolysis of tea waste (detailed preparations of sediments and biochar were provided in Supplementary material).

### 2.2. Plant growth

Ramie seedlings were purchased from the Ramie Institute of Hunan agriculture university, Changsha, China. Before transplanting, different amounts of TB were added into and mixed up with the Cd contaminated sediments to obtain 100, 500, 1000 and 5000 mg $kg^{-1}$ biochar contained Cd contaminated sediments. Then, ramie seedlings were transplanted into the above sediments and cultivated in a growth chamber for two months. The cultivation conditions were 14 h photoperiod (6:00 a.m. to 8:00 p.m.), $24 \pm 5$ °C/$18 \pm 3$ °C day/night temperature, and $65 \pm 5\%$ relative humidity. All the treatments were conducted in 4 replicates. Cd contaminated sediments without the addition of biochar were used as control. Based on the concentrations of TB, treatments were abbreviated as TB100, TB500, TB1000 and TB5000, while CK was referred to the control treatments.

### 2.3. Metal analysis

To assess the behavior of Cd in the sediments, the metal speciation of Cd in the day of 0, 7, 15, 30 and 60 were measured using the BCR (European Community Bureau of Reference) sequential extraction procedure described by Wan et al. (2016) (detailed method was provided in Supplementary Material).

To assess Cd accumulation and translocation in ramie seedlings, ramie leaves, stems and roots were collected separately and rinsed completely with ultrapure water after two months of cultivation. The washed plant organs were dried in oven at 70 °C till constant weight and digested with $HNO_3$ and $HClO_4$ (3:1, v/v) using a graphite digestion apparatus (DS-360). The digested liquid was used to determinate Cd concentration by the atomic absorption spectrometer (PEAA700, PerkinElmer, U.S.A.). Cd translocation factor (TF) was calculated as follows:

$$TF = \frac{Cd_L \times B_L + Cd_S \times B_S}{(B_L + B_S) \times Cd_R}$$

where $Cd_L$, $Cd_S$, and $Cd_R$ referred to the concentration of Cd in ramie leaves, stems and roots, respectively. $B_L$ and $B_S$ represented the dry biomass of ramie leaves and stems, respectively.

In order to further investigate the influence of biochar on the behavior of Cd in plant cells, the subcellular distribution of Cd in ramie leaves, stems and roots were determined using the differential centrifugation technique as described previously (detailed method was provided in Supplementary Material) (Gong et al., 2017b).

### 2.4. Plant toxicity analysis

To assess the effect of biochar on plant growth, the biomass of ramie seedlings were determined. After two months of cultivation, ramie leaves, stems and roots were collected separately and washed with ultrapure water thoroughly. Then, the different ramie organs were dried in oven (70 °C) till constant weight and cooled down to room temperature to determine the dry biomass.

The contents of hydrogen peroxide ($H_2O_2$) and malondialdehyde (MDA) in ramie seedlings were determined to explore the roles of biochar in plant oxidative damage. $H_2O_2$ content was measured by the potassium iodide spectrophotometric method described by Velikova et al. (2000). MDA concentration was determined following the thiobarbituric acid (TBA) method described by Chaoui et al. (Chaoui et al., 1997). The unit of $H_2O_2$ or MDA was nmol $g^{-1}$ FW (fresh weight).

### 2.5. Microbial activity determination

Enzyme activities in the Cd contaminated sediments can be used as the indicators of microbial activity. To assess the effects of biochar on the microbial activity in metal contaminated sediments, the activity of urease, phosphatase, invertase and catalase in the day of 0, 7, 15, 30 and 60 were determined (detailed methods were provided in Supplementary Material). Urease was analyzed following the sodium phenolate-sodium hypochlorite colorimetric method described by Hu et al. (2014). The activity of urease was expressed as mg $NH_3$-N $g^{-1}$ sediments 24 $h^{-1}$. Phosphatase was measured following the disodium phenyl phosphate colorimetric method described by Jin et al. (2016). The activity of phosphatase was expressed as mg phenol $g^{-1}$ sediments 24 $h^{-1}$. Invertase was determined by the 3,5-dinitrosalicyclic acid colorimetric method described by Chen et al. (2013). The activity of invertase was expressed as mg glucose $g^{-1}$ sediments 24 $h^{-1}$. Catalase in the sediments was measured by the potassium permanganate ($KMnO_4$) titration method described previously (Huang et al., 2016). The activity of catalase was expressed as mL $KMnO_4$ $g^{-1}$ sediments 20 $min^{-1}$.

### 2.6. Statistical analysis

Results were presented as means ± standard errors. Statistic Package for Social Science (SPSS) was applied to analyze the data. Duncan test was carried out to analyze the statistical significance among different treatments at a probability level of $p < 0.05$.

## 3. Results and discussion

### 3.1. Characterizations of sediments and biochar

See Supplementary material.

### 3.2. Metal behavior influenced by biochar

#### 3.2.1. Metal speciation in the contaminated sediments

Metal speciation influence metal behavior and its toxicity in the sediments. In this study, four step BCR method was used to investigate the effect of biochar on the change of Cd speciation in river sediments where ramie seedlings were growing. According to this method, metals can be classified into four fractions, and the mobility and bio-toxicity of metals decreased in the order of acid-soluble fraction > reducible fraction > oxidizable fraction > residual fraction (Begum et al., 2012; Gao et al., 2010). The percentage of Cd in different fractions was shown in Fig. 1. TB changed the speciation of Cd in the early stage. On the 4th day, TB promoted the transformation of Cd from residual fraction to other forms, and it increased the acid soluble fraction of Cd by 7.04–12.16%. However, the percentage of Cd in different speciation tended to be stable with the increase of incubation time. It is worth noting that TB increased the reducible fraction of Cd in all the treatments compared with control, whereas the residual fraction of Cd only increased in the TB5000 treatment on day 7 and 60.

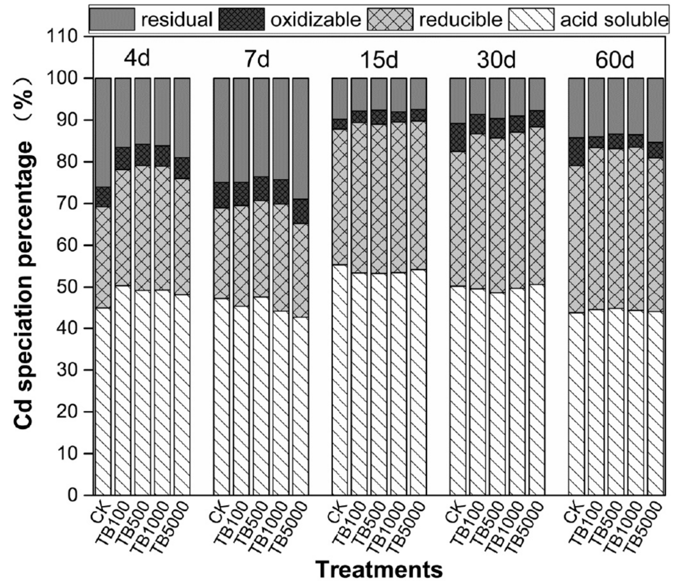


**Fig. 1.** Effects of TB on the change of Cd speciation in the contaminated river sediments growing with ramie seedlings. CK, TB100, TB500, TB1000 and TB5000 denote 0, 100, 500, 1000 and 5000 mg $kg^{-1}$ TB treatments.

The acid soluble fraction of metals mainly refers to metals that in ionic form or band to carbonate, which is also known as the exchangeable fraction and can be easily bioaccumulated in the organisms. Different from what was observed in this study, the acid soluble fraction of Cd in the contaminated soil decreased by 24–32%, 19–23%, and 22–27% with the addition of biochar derived from rice straw, rich hull and maize stover, respectively (Bashir et al., 2018). A similar decrease was also observed by Lu et al. using bamboo derived biochar at the rates of 1% and 5% (Lu et al., 2017). They ascribed these effects to the immobilization of metals by biochar. However, the incorporation of TB at early stage increased the acid soluble fraction of Cd in this study, which might due to the interaction between biochar and the root exudates. When adding TB to the contaminated sediments where ramie seedlings were cultivating, it is hypothesized that the introduction of TB might interact with root exudates to form carbonate, and then banding to Cd, thus increasing the cadmium carbonate proportion in the contaminated sediments. As for the reducible fraction, which mainly consist metals bounding to iron and manganese oxides or hydroxides, the presence of iron, oxygen and hydrogen on TB might lead to the formation of iron oxides or hydroxides with Cd, thereby increasing the reducible fraction of Cd in the contaminated sediments. Besides, biochar is alkaline under normal conditions, therefore the addition of biochar generally could increase the pH of sediments, which perhaps could promote the formation of metal hydroxides and increase the reducible metal portion. Similarly, Jiang et al. reported that the reducible portion of Cd, Cu and Pb increased obviously in a polluted Ultisol treated with 3% and 5% biochar (Jiang et al., 2012). The acid soluble and reducible fractions of metals are considered as the loosely bound phases, whereas the other two fractions, especially the residual portion, are the stable fractions, which are inaccessible to organisms (Gong et al., 2018b). Biochar was reported to increase the oxidizable and residual fractions of metals in soil due to their high adsorption affinity and unique physicochemical property (Zeng et al., 2018). Nevertheless, in the end of incubation (day 60), TB merely caused a slight decrease in the percentage of oxidizable Cd in the contaminated sediments, and only the TB5000 treatments slightly increased the residual portion of Cd in the contaminated sediments compared with control. These extraordinary consequences may result from the relative low application contents of biochar and the interaction between biochar and plants.

#### 3.2.2. Metal behavior in the plants influenced by biochar

The influence of TB on the bio-accumulation and translocation of Cd in ramie seedlings was shown in Fig. 2A. The application of TB increased Cd concentration in ramie roots compared with control, with the exception of the TB5000 treatments, in which the concentration of Cd decreased significantly. Similar to what was observed in roots, TB at 100, 500 and 1000 mg $kg^{-1}$ increased Cd concentration in ramie stems by 12–20%, whereas the 5000 mg $kg^{-1}$ TB reduced Cd concentration by 5% relative to the control. However, no statistical difference in Cd concentration was observed in ramie leaves whether the seedlings were treated with TB or not. The TF value of Cd in ramie seedlings increased with increasing the concentration of TB (Fig. 2A). In addition, the subcellular distribution of Cd in ramie seedlings was influenced by the application of TB (Fig. 2B). TB increased the organelle portion of Cd in ramie roots, stems and leaves. Notably, in ramie stems, relative to the control, the cell wall fraction of Cd decreased and the organelle portion of Cd increased with the application of TB at 100, 500 and 1000 mg $kg^{-1}$.

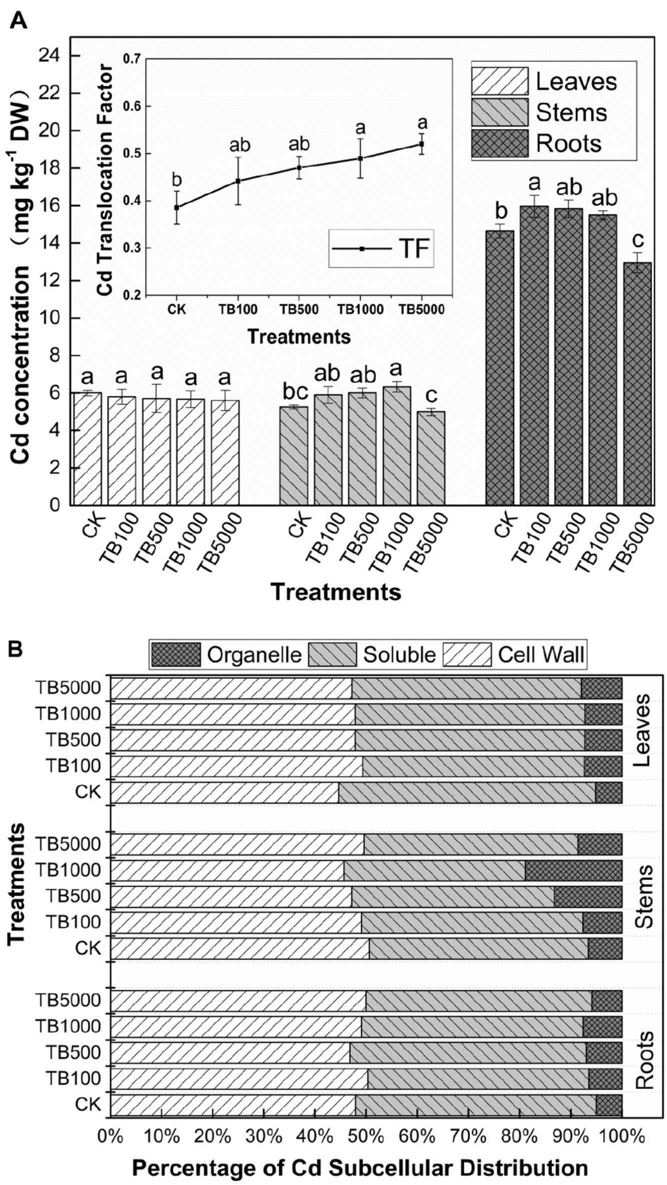


**Fig. 2.** Cd behavior in the plants changed by TB. A corresponds to the Cd concentration and Cd translocation factor in ramie seedlings. B corresponds to the subcellular distribution of Cd in ramie seedlings. CK, TB100, TB500, TB1000 and TB5000 denote 0, 100, 500, 1000 and 5000 mg $kg^{-1}$ TB treatments. Data are means ± standard error of four replicates. Different letters (a, b, c) above the error bars indicate significant differences among various concentrations of TB treatments ($p < 0.05$) analyzed by Duncan test.

Biochar is capable of reducing the bioavailability of heavy metals to plants. For example, Xu et al. reported that biochar derived from bamboo reduced Cd concentration in maize shoots by 50.9% (Xu et al., 2016). Lu et al. found that the accumulation of Cd, Cu, Pb and Zn by *Sedum plumbizincicola* cultivating in the biochar amended soil was lower by the precipitation and adsorption of heavy metals to biochar (Lu et al., 2014). However, in the present study, the higher Cd concentration in ramie stems and roots was found in the 100, 500 and 1000 mg $kg^{-1}$ TB treatments compared with control, which may be due to the increased Cd percentage in the acid soluble and reducible forms at the early stage of these treatments. Besides, the increased organelle Cd percentage indicated the promoted entrance of Cd into ramie cells by TB, which perhaps also accounted for the increased Cd concentration at these treatments. Cd is a non-essential heavy metal and to our knowledge there are no specific channels or transporters for Cd in the plants. Cd was supposed to get into plants through the transportation corridors for essential elements. As reported previously, biochar was able to change the concentrations of essential elements in soil. For example, a study conducted by Mukheriee and Zimmerman showed that laboratory-produced biochar could release nutrients to the amended soil (Mukherjee and Zimmerman, 2013). Houben et al. demonstrated that the concentrations of available nutrients, such as calcium (Ca), potassium, magnesium (Mg) and phosphorus (P), were increased due to the presence of these nutrients in biochar (Houben et al., 2013). In this work, the presence of iron (Fe), Ca, and Mg on the biochar probably will stimulate the transporters for essential elements uptake, which sequentially promoted the uptake and translocation of Cd in the plants and facilitated the entrance of Cd into ramie cells. What is noticeable is that the TB5000 treatments reduced Cd concentration in ramie roots and stems compared with control. And these results were similar to what was observed in many previous studies (Al-Wabel et al., 2015; Fellet et al., 2014). The different results obtained among TB treatments may due to the different application concentrations. TB at relative high contents (≥5000 mg $kg^{-1}$) was likely to presence a strong adsorption capacity and immobilizes Cd and other nutrients in the sediments, therefore reducing the bioavailability of metals.

### 3.3. Plant toxicity modified by biochar

#### 3.3.1. Plant growth influenced by biochar

Plant growth inhibition is the most direct and obvious symptom in plants under heavy metal stress. As shown in Fig. 3A, relative to the control, TB increased the dry biomass by 32–46%, 23–33%, and 11–18% in ramie leaves, stems and roots, respectively, indicating the alleviation of heavy metal induced toxicity by TB in the plants. The highest dry biomass of leaves and stems was observed in ramie seedlings treated with 500 mg $kg^{-1}$ TB, while that of roots was found in the TB100 treatments, suggesting that TB at low levels was more efficient in the promotion of plant growth. Similar to what was observed in the present study, the addition of biochar to soil significantly stimulated the plant growth in *Jalropha curcas* L. and *Brassica juncea* (Jin et al., 2011; Suppadit et al., 2012). The promoted plant growth might due to the water and nutrients retention and the improved sediments physical and chemical properties (e.g. CEC, pH, TOC). In addition, Spokas et al. demonstrated that biochar could produce ethylene, a kind of plant hormone, in the soil, and this also might be one of the mechanisms for the growth promotion by TB in this study (Spokas et al., 2010).

#### 3.3.2. Oxidative stress modified by biochar

The overproduction of reactive oxygen species (ROS) caused by Cd in plants could trigger oxidative damage to proteins, DNA and lipids, and thus may lead to tissue necrosis or ultimately kill the plants

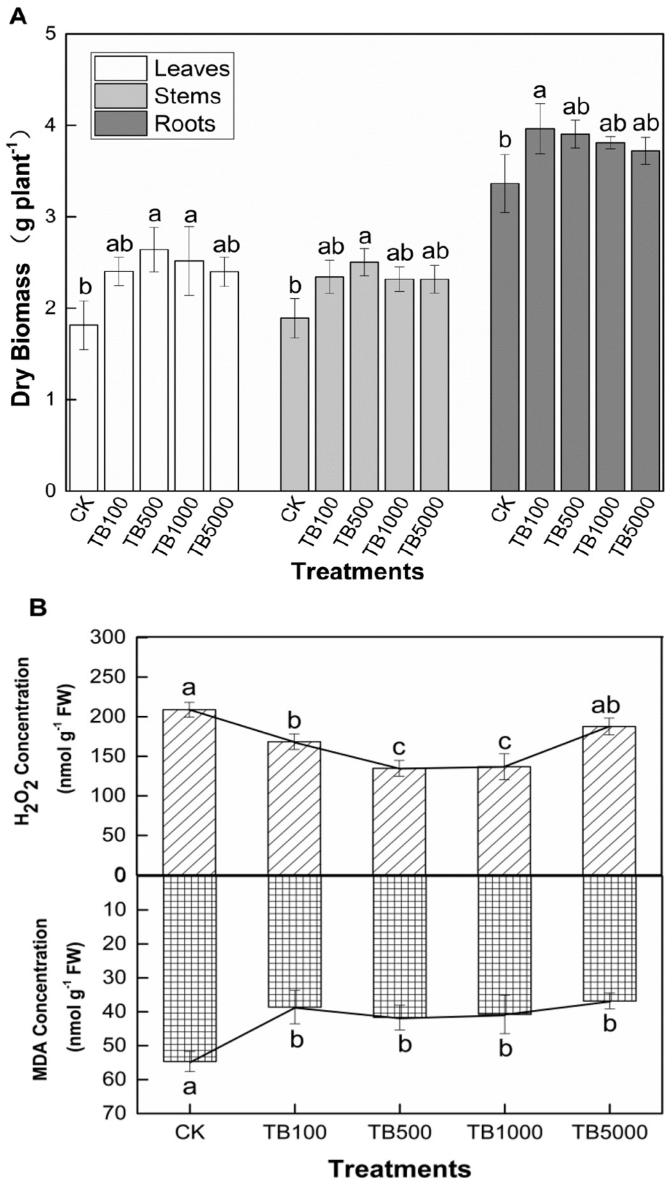


**Fig. 3.** Plant toxicity regulated by TB. A and B correspond to the dry biomass (A) and $H_2O_2$ and MDA accumulation (B) in ramie seedlings, respectively. CK, TB100, TB500, TB1000 and TB5000 denote 0, 100, 500, 1000 and 5000 mg kg$^{-1}$ TB treatments. Data are means ± standard error of four replicates. Different letters (a, b, c) above the error bars indicate significant differences among various concentrations of TB treatments ($p < 0.05$) analyzed by Duncan test.

(Martinez Dominguez et al., 2010; Schutzendubel and Polle, 2002; Verma et al., 2013). In this study, MDA and $H_2O_2$ were used as the indicators of oxidative damage in the plants. MDA is the product of lipid peroxidation, while $H_2O_2$ is a typical ROS. Their excess accumulation indicates oxidative damage in the plants. As shown in Fig. 3B, TB decreased the content of MDA in ramie seedlings by 24–33% compared with control. However, MDA content did not changed significantly under various concentrations of TB treatments. The concentration of $H_2O_2$ also decreased with the application of TB. The lowest $H_2O_2$ content (134.71 nmol g$^{-1}$ FW) was observed in ramie seedlings treated with 500 mg kg$^{-1}$ TB. $H_2O_2$ content in the TB5000 treatments was lower than control, but it was significantly higher than that observed in ramie seedlings treated with 500 and 1000 mg kg$^{-1}$ TB. The reduced contents of MDA and $H_2O_2$ observed in TB treatments indicated the alleviation of Cd induced oxidative damage by TB in ramie seedlings. These findings are consistent with previous studies. Younis et al. reported that biochar ameliorated Cd induced toxic effects in *Spinacia oleracea* by promoting plant growth, up-regulating protein content, and decreasing the MDA content (Younis et al., 2016). Abbas et al. found that rice straw derived biochar decreased Cd induced oxidative stress in wheat through reducing the accumulation of MDA and $H_2O_2$ (Abbas et al., 2017).

The alleviation of heavy metal induced oxidative damage in plants by biochar has been confirmed by previous reports, and many of them ascribed this alleviation to the reduction of metal accumulation (Younis et al., 2015a; Younis et al., 2015b), which could also explain the alleviated toxic effects in the TB5000 treatments in this study. However, the application of TB at relative low levels increased the accumulation of Cd in ramie seedlings, but a remarkable decrease in the MDA and $H_2O_2$ contents was observed in those treatments. Besides, the minimum Cd concentration was observed in the TB5000 treatments, while the lowest $H_2O_2$ content did not appear in these treatments. Thus, the reduction of metal accumulation is only partly the cause of biochar induced toxicity alleviation in the plants. Biochar was reported to accelerate •OH, $SO_4•^-$ and $O_2•^-$ participated radical oxidation process due to its specific surface areas, defective sites and the existence of functional groups (Tang et al., 2018). Fang et al. found that biochar activated $H_2O_2$ by the persistent free radicals observed on the surface of biochar (Fang et al., 2014). The functional groups and free radicals released from biochar perhaps could account for the ROS scavenging in the plants. In addition, Mehari et al. reported that biochar-mediated induced-resistance in tomato involved defense-related gene expression which was correlated with ROS accumulation (Mehari et al., 2015). The gene regulation by biochar might result in the alleviation of metal induced oxidative damage by biochar. In addition, compared with the 500 and 1000 mg kg$^{-1}$ TB treatments, TB at 5000 mg kg$^{-1}$ significantly increased $H_2O_2$ content in ramie seedlings, suggesting that the alleviation of metal induced toxicity by biochar was concentration dependent and biochar at low levels was more efficient in mitigating Cd induced oxidative damage in plants. Further detailed studies are needed to explore the exact mechanisms on how can different concentrations of biochar affect heavy metal induced oxidative damage in the plants.

### 3.4. Microbial activity changed by biochar

Enzymes activities in the sediments are considered to serve as the indicators of microbial activity in this study. The activities of urease, phosphatase, catalase and invertase changed by TB during 60 days of incubation were shown in Fig. 4. In Cd contaminated river sediments, the activities of urease and phosphatase changed in a similar pattern: decreased to the 30th days and then to be stable. Inversely, the invertase activity increased gradually and peaked on day 60. The activity of catalase kept stable to the 30th days and then it increased. The addition of TB significantly increased the activities of urease and phosphatase at the earlier days of incubation, but these increases leveled off on day 60, whereas the activity of invertase was decreased by TB with increasing the incubation time. As for catalase, TB slightly increased its activity in all the treatments compared with control.

The changes of enzymes activities reflected the dynamic changes of microbial activity in the 60 days of incubation. Previous studies have confirmed the negative correlations between heavy metals concentration and soil microbial activity (Huang et al., 2015). In the present study, with the incubation time increase, the concentration of Cd in the sediments decreased due to the bioaccumulation of Cd by ramie seedlings, which might increase the microbial activity and enzymes activities in this Cd contaminated sediments. This could be one of the reasons for the increased activities of invertase and catalase with increasing the incubation time. However, the activities of urease and phosphatase decreased during the incubation process. Urease and phosphatase are capable of governing the dynamics of nitrogen (N) and P cycling in the environment, and these elements are essential nutrients for plant growth and development. The uptake of nutrients by ramie seedlings

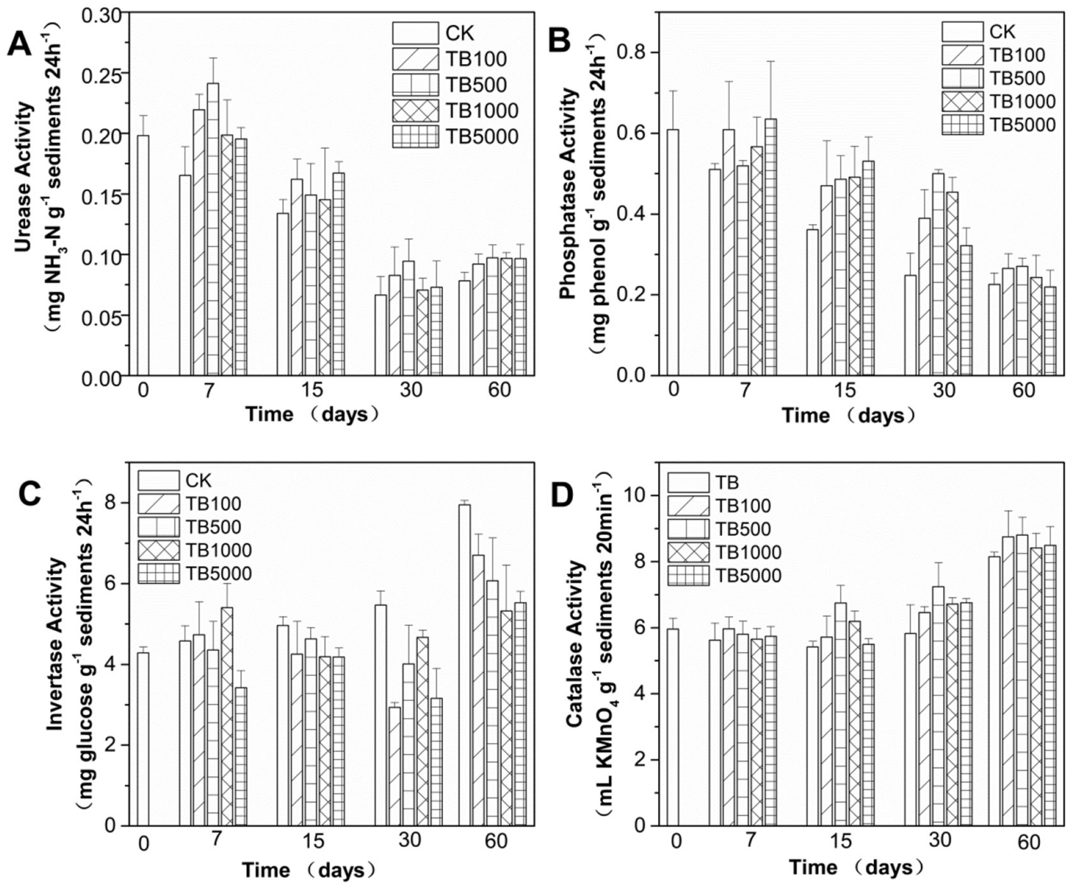


**Fig. 4.** Effects of TB on the activities of urease (A), phosphatase (B), invertase (C) and catalase (D) in the Cd contaminated river sediments cultivating with ramie seedlings. CK, TB100, TB500, TB1000 and TB5000 denote 0, 100, 500, 1000 and 5000 mg $kg^{-1}$ TB treatments. Data are means ± standard error of four replicates.

might reduce the sources of N and P for microbes, thus resulting in the activities decrease of urease and phosphatase in this study.

The regulation of microbial activity in the contaminated environment by biochar has been demonstrated in a variety of reports (Bashir et al., 2017; Nie et al., 2018). In this study, TB increased the activities of urease, phosphatase and catalase, indicating the promotion of microbial activity by TB in heavy metal contaminated environment. These findings are in accordance with previously published studies. Gong et al. demonstrated that the inoculation of bamboo biochar gave rise to greater microbial counts and activity (Gong et al., 2018a). Ahmad et al. found that the abundance of bacteria, fungi and actinomycetes increased in the heavy metal contaminated agricultural soil treated with biochar derived from soybean stover and pine needle (Ahmad et al., 2015). The promotion of microbial activity by TB in ramie planting Cd contaminated sediments could be explained by two reasons. One is that TB could provide a better habitat for the microbial communities due to the high surface area of biochar and its role in the changing of sediment physical and chemical property which was supported by the study of Lu et al. (2015). The other is that the supplementary of nutrients (e.g. carbon source, N source, and minerals) that are necessary for microbe growth by the inoculation of TB would help increase or maintain the microbial activity under Cd stress. It is worth noting that the activities of urease, phosphatase, and catalase peaked at TB500 treatments on the day of 30 and 60, indicating that TB at low levels was more efficient in the promotion of microbial activity. Similar to what was observed in this study, Bhaduri et al. found that the highest activities of dehydrogenase and phosphatase were observed in the lowest rate of biochar treatments (Bhaduri et al., 2016). Biochar at high contents was also reported to decrease microbial activity due to its detrimental effects to microbes (Huang et al., 2017b). Since the secretions and degradation products produced by microbes could influence metal behavior and plant growth, the promoted microbial activity under low levels of TB treatments might account for the change of Cd speciation and the promotion of plant growth observed in these treatments. Besides, different from what was observed in urease, phosphatase, and catalase, the activity of invertase was decreased by TB, suggesting that the influences of TB on enzymes activities were enzyme dependent and TB was beneficial for the growth of urease-, phosphatase-, and catalase-producing microbes in the Cd contaminated sediments.

## 4. Conclusion

In this study, TB, a biochar derived from the pyrolysis of tea waste, was prepared to facilitate the phytoremediation of Cd contaminated sediments. Results showed that TB at 100, 500 and 1000 mg $kg^{-1}$ could promote the transformation of Cd from other forms to reducible fraction in sediments and enhance the entrance of Cd from cell wall and the soluble parts to the organelle, thus facilitating the accumulation and translocation of Cd in ramie seedlings. Moreover, TB alleviated Cd induced growth inhibition and oxidative damage in ramie seedlings by reducing ROS accumulation. The activities of urease, phosphatase and catalase in the Cd contaminated river sediments were promoted by TB, indicating the mitigation of Cd induced toxicity to microbes. These results suggested that biochar at low levels was beneficial to the phytoremediation of Cd contaminated sediments, and it could mitigate Cd induced toxic effects to plants and microbes.

## Acknowledgments

This study was financially supported by the Program for the National Natural Science Foundation of China (51879101, 51579098, 51779090, 51709101, 51521006, 51809090, 51278176, 51378190, 51809090), the National Program for Support of Top–Notch Young Professionals of China (2014), the Program for Changjiang Scholars and Innovative Research Team in University (IRT-13R17), and Hunan Provincial Science and Technology Department Plan Project (2018SK20410, 2017SK2243, 2016RS3026), and the Fundamental Research Funds for the Central Universities (531109200027, 531107051080, 531107050978).

## Declaration of interest

None.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2019.02.215.

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