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Bioremediation of cadmium polluted soil using a novel cadmium immobilizing plant growth promotion strain *Bacillus* sp. TZ5 loaded on biochar

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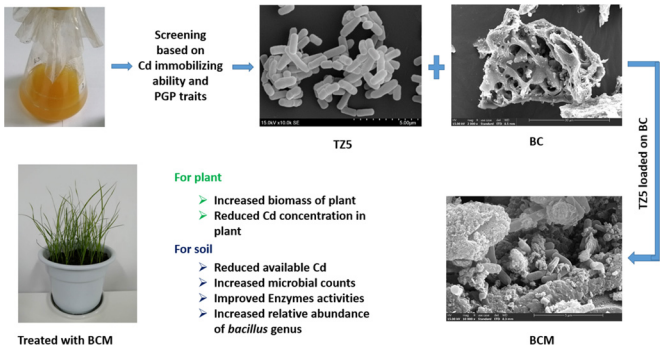
GRAPHICAL ABSTRACT



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ABSTRACT

Bioremediation of cadmium polluted soil using biochar (BC) and plant growth promotion bacteria (PGPB) have been widely concerned. In our study, a novel Cd immobilizing PGPB strain TZ5 was isolated based on the Cd immobilizing potential and plant growth promotion (PGP) traits. Further, changes of surface morphology and functional groups of TZ5 cells were observed after exposed to Cd^{2+} by SEM-EDS and FTIR analyses. Then, the strain TZ5 was successfully loaded on BC as biochemical composites material (BCM). Pot experiment indicated that the percentage of acetic acid-extractable Cd in BCM treatments significantly decreased by 11.34 % than control. Meanwhile, BCM significantly increased the dry weight of ryegrass by 77.78 %, and decreased the Cd concentration of ryegrass by 48.49 %, compared to control. Microbial counts and soil enzyme activities in rhizosphere were both significantly improved by BCM. Furthermore, the proportion of relative abundance of *Bacillus* genus was enhanced after treated by BCM, which indicated that the strain TZ5 was successfully colonized in the rhizosphere. This study provided a practical strategy for bioremediation of Cd contaminated soil.

1. Introduction

Heavy metal pollution is posing a significant hazard to the ecosystem and the public health due to its toxicity, bioaccumulation and non-biodegradability (Zeng et al., 2019). Cadmium (Cd), one of the most toxic heavy metals, is class-I carcinogen as well as a chronic potent

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nephrotoxin (Meharg et al., 2013; Zeng et al., 2015). A nationwide investigation reveals that Cd is the most frequently detected heavy metal in farmland, and 7 % of the investigated sites are contaminated by Cd according to the standards for soil in China (Xiao et al., 2017). Hence, it's extremely essential and urgent to develop effective, eco-friendly and low-cost technologies to remediate Cd polluted soil.

In situ immobilization of Cd in soil is regarded as a practicable and cost-effective technology, because it does not need to stop agricultural production activities during the remediation progress. Heavy metal immobilization is usually achieved by *in situ* application of chemical amendments or microorganisms, which are effective both in heavy metal immobilization and plant growth promotion (PGP). Compared to chemical sorbents, the inoculation of microorganisms is more environment-friendly, due to attenuating the toxic influence of Cd on living beings without creating secondary pollution and destroying soil properties (Jin et al., 2011; Ye et al., 2020). And bioremediation using functional microorganisms as "immobilizing agent" is really practicable to vast Cd contaminated areas, e.g., agricultural soil (Khan et al., 2004; Wang et al., 2014a).

In last few decades, it has been reported that some types of plant growth promoting bacteria (PGPB) are of high resistibility to Cd, and they can diminish Cd bioaccumulation in plants through precipitating or absorbing Cd. These Cd resistant PGPB strains with high Cd immobilizing ability include *Pseudomonas* (Li et al., 2018), *Delftia* (Liu et al., 2020), *Enterobacter* (Mitra et al., 2018; Pramanik et al., 2018), *Arthrobacter* (Bafana et al., 2010), *Bacillus* (Wang et al., 2014a; Jiang et al., 2009) etc. Since the selected strains may not be able to perform well in diverse contaminated sites, there is still a crying need to screen and isolate newer Cd immobilizing PGPB strains. As a result of long periods of domestication locally, we can screen out bacteria that could adapt to the local environment and also immobilize heavy metals.

Another main limitation of bioremediation efficiency is that the contaminated soil usually can't support the rapid growth of the inoculated bacteria, on account of the nutritional deficiency and competition by other microorganisms (Reddy et al., 2003). Therefore, a strategy to improve the survival and distribution of inoculum is to provide microorganisms with carrier materials. Nevertheless, the usual carrier materials often have limitations that restrict their widespread application. For example, vermiculate and peat sometimes are unavailable in areas where they do not exist naturally, what's more, unearthing these materials would has a negative environmental influence (Herrmann and Lesueur, 2013; Wu et al., 2019a). Biochar (BC) achieved by the pyrolysis process has properties that are conducive for use as bacterial carrier material, including large specific surface area, high internal porosity and the affinity to bacteria (Xiao et al., 2017; Hale et al., 2014; Wu et al., 2020). Moreover, researchers have indicated that BC contains plentiful nutrients (C, K, N and P etc.), and can be as slow-release fertilizers, bringing long-term advantages to the survival of microbes (Masiello et al., 2013; Ye et al., 2019). Accordingly, we conclude that microorganisms loaded on BC as biochemical composites material (BCM) could be an innovative idea to improve the efficiency of bioremediation.

Hence, we screened and isolated a Cd immobilizing PGPB strain, and characterized the morphology and functional groups of the isolated strain by scanning electron microscopy (SEM), energy dispersive spectrometer (EDS) and Fourier transform infrared spectra (FTIR). Then, we designed a novel biochemical composite material using biochar as inoculum carriers. The performance of BCM on bioavailability of Cd and soil biochemical properties were comprehensively evaluated. Besides, the changes of soil bacterial community structure were tested by high throughput sequencing.

2. Materials and methods

2.1. Isolation and identification of Cd immobilizing PGPB strains

The Cd resistant strains were isolated from Cd polluted agricultural soil collected from Guangyuan, China (105°54′08.21″N, 32°35′56.30″W), and the main characteristics of soil were shown in Table S1. For isolation, 10 g soil was suspended in 100 mL of 0.85 % normal saline, then shaking the slurry for 30 min. The extraction solution was serially diluted and spread on Luria-Bertani (LB) plates added with the increasing concentration of Cd (up to 150 mg/L), and incubated at 37 °C for 7 d. The well-grown and morphologically different colonies were re-streaked several times to obtain pure isolates.

To test whether the Cd resistant strains selected had Cd immobilizing potential, these Cd resistant strains were inoculated into the original contaminated soil respectively. Each pot contained 1.0 kg of the original contaminated soil, then every pot was sprayed with 20 mL bacteria suspension of logarithmic phrase ($\sim 10^9$ CFU/mL). While 15 mL of sterilized deionized water was used as a control. All these pots received unified water management (Li et al., 2016). After incubation for 15 d, the available Cd in soil was extracted by 0.1 mol/L $CaCl_2$ (Pueyo et al., 2004). To be specific, 5 g dry soil were shaken for 2 h at 25 ℃ with 25 ml of 0.1 mol/L $CaCl_2$. The suspension was centrifuged at 3000 r/min for 10 min and then was filtered by 0.45 μm filter film. And the concentration of Cd was determined by atomic absorption spectroscopy (AAS; VARIAN, SpecterAA-220Fs, USA) (Li et al., 2019a).

Then the isolates with the ability to immobilize Cd were tested to evaluate their PGP traits. The IAA production and phosphate solubilizing substances were qualitatively measured as described by Bric et al. (1991), and Fiske and Subbarow (Fiske and Subbarow, 1925), respectively. Based on the Cd immobilizing potential and PGP traits, the dominant strain was selected as the target Cd immobilizing PGPB strain.

The target strain was identified by 16S rDNA sequencing (Byers et al., 1998) and the similar DNA sequences were matched in GeneBank database using BLAST.

2.2. Characterization of strain TZ5

SEM (JSM-7500 F, Japan) with EDS microanalysis system was applied to observe the changes of surface morphology and surface elemental composition of the target strain cells after Cd^{2+} treated (10 mg/L) for 24 h at 37 ℃. Besides, the changes of functional groups of the target strain cells after Cd^{2+} treated were measured by FTIR (Nicolet 6700, USA). Samples for analysis were processed according to previous description (Pramanik et al., 2018).

2.3. Preparation and characterization of BCM

Coconut shell biochar used in the present study was achieved from Desheng Active Carbon Factory (Jiangsu, China) and the production condition was pyrolysis at about 800 ℃, with a retention time of 6 h (Liu et al., 2018). To prepare BCM, the immobilization of the target strain on biochar was conducted in the optimal condition, which was ascribed in our previous study (Xiao et al., 2017). In Brief: the 100 mL bacteria suspension of logarithmic phrase ($\sim 10^9$ CFU/mL) and 5 g BC were mixed with a ratio of 20:1 (v:w) and shaken at 37 ℃ for 12 h with 160 rpm. Both non-immobilized and immobilized TZ5 were rinsed with DI water and stored in suspension at 4 °C until use. SEM was used to compare the morphologies of BC and BCM to verify the fixed effect of bacteria. And the changes of surface functional groups of BC and BCM were also evaluated by FTIR.

2.4. Pot experiment

The plastic pots containing 2 kg Cd polluted soil were used for this

pot experiment. The amounts of these different biochemical materials added were 100 mL of BC (5 g) suspension (T1), 100 mL of bacteria suspension (T2), and 100 mL of BCM suspension (T3). The polluted soil amended with 100 mL of 0.85 % sterilized normal saline was as control (CK).

Ryegrass (*Lolium perenne*) seeds were previously sterilized as described by Benizri et al. (Li et al., 2016). Fifty seeds were sown per pot. All these treatments were replicated three times and performed unified water and temperature management. And the temperature range was 25–27 °C during day and 20–22 °C during night. After germination, thirty healthy and uniform sprouts of ryegrass were reserved. Continuing incubation for about eight week, before the plants were harvested. The plants samples were washed with DI water carefully, and dried at 60℃ in an oven, then the dry weight (DW) was recorded.

2.5. Heavy metal and soil pH analysis

The ryegrass samples and dried soil samples from different treatments and CK were grinded in a mortar (< 0.25 mm). Then, the powered plant samples (0.1 g) were digested with the mixed solution of HNO_3, $HClO_4$ and HF (5:4:3, v/v) as described by Liu et al. (2015). Metal speciation in soil was performed by a BCR sequential extraction process (Kartal et al., 2006), and the method defined Cd in four chemical forms: acetic acid-extractable (HOAc-extractable), reducible, oxidizable, and residual speciation. The contents of Cd in ryegrass and soil were also determined by AAS. Soil pH was evaluated in a soil/water slurry at a 1:2.5 (w/v) ratio.

2.6. Numbers of microbes and activities of soil enzymes

The rhizospheric soil samples of ryegrass were collected carefully. The numbers of microbes and the activities of soil enzymes were evaluated to reflect soil biochemical qualities. The numbers of microbes were conducted by the spread plate count method according to previous report (Liu et al., 2018), and the numbers of colony forming units (CFU) of bacteria and fungi were counted. Dehydrogenase activity was determined by the amount of triphenylformazan (TPF) at the wavelength of 492 nm and defined as mg TPF/g soil/24 h (Benefield et al., 1977). Acid phosphatase activity was evaluated the content of p-nitrophenol (pNP) release at the wavelength of 400 nm and expressed as mg pNP/g soil/h (Aarle and Plassard, 2010). Urease activity was spectrophotometrically measured by an NH_4-N coloured complex at 578 nm and conveyed as μg NH_4-N/g soil/24 h (Yan et al., 2013).

2.7. Bacterial community

The soil DNA was extracted using a Soil DNA Kit (Omega Biotek Inc., Norcross, GA) according to the manufacturer's protocols. Afterwards, the 16S rRNA gene was amplified with primers 338 F (5′-ACTCCTACGGGAGGCAGCAG-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′) by thermocycler PCR system (GeneAmp 9700, ABI, USA). The bacterial community was analyzed on the Illumina MiSeq platform, which was conducted by Majorbio Bio-Pharm Technology Co., Ltd (Shanghai, China).

2.8. Data analysis

All experiments were carried out in triplicates. Results were evaluated with one-way ANOVA at a significance level of $P \leq 0.05$.

3. Results and discussion

3.1. Isolation and identification of TZ5

In this study, twelve strains with the ability to resist 150 mg/L Cd were isolated. As shown in Table S2, concentration of available Cd in soil after incubation with five of the strains (TB3, TB4, TZ1, TZ4 and TZ5) decreased than CK, and concentration of available Cd in soil treated by TZ5 was the lowest in all treatments. Thus, the strain TZ5 possessed the highest Cd immobilizing ability. Meanwhile, the phosphate solubilizing capacity, and IAA production of these five Cd-immobilizing PGPB were measured (Table S3). The phosphate solubilizing capacities of the five strain were at the level of 63.72–161.23 mg/L, and TZ5 presented the better phosphate solubilizing capacity than other strains. It was reported that phosphate solubilizing bacteria could increase soluble phosphorus to promote plant growth as well as minimize Cd mobilization to plants (Pramanik et al., 2018; Jinhee et al., 2010). Moreover, those isolates produced IAA at the level of 10.16–45.85 mg/L, especially for the strain TZ5 and strain TZ4 whose productions were all over 40 mg/L. Compared to other reports, these productions of strain TZ5 are relative higher than those produced by some of PGPB (Prapagdee et al., 2013; Ma et al., 2016; Han et al., 2017).

In view of the capabilities of Cd tolerance and immobilization as well as PGP traits, the strain TZ5 was selected for further research. Comparison of 16S rDNA sequence of in NCBI GenBank, the strain TZ5 was identified as *Bacillus* sp. (NCBI number: MN629179), and its sequence was listed in Table S4.

3.2. Characterization of TZ5

3.2.1. SEM-EDS analysis

To explore possible mechanisms into the Cd immobilizing capability of TZ5, the

Cd^{2+} treated TZ5 was studied by SEM-EDS. Without Cd^{2+} treated, TZ5 appeared to be short rods with smooth surface (Fig. 1a), and there was no Cd responsible peak detected (Fig. 1c). After Cd^{2+} exposure, TZ5 seemed to be longer and thinner, and there was large amount of shiny floccus on the surface of TZ5 (Fig. 1b). Furthermore, EDX spectra presented that there were some characteristic Cd peaks (Fig. 1d). The existence of Cd element demonstrated the adsorption or deposition of Cd on the surface of TZ5. Thus, the morphological changes of TZ5 seemed to be associated with the adsorption of Cd, which might play a crucial part in the interaction of Cd and bacterial surface as well as the tolerance mechanism to Cd toxicity, and such observations were also found by other researchers (Wang et al., 2014b; Huang et al., 2013). Moreover, the element mapping showed that the surface of Cd^{2+} treated TZ5 was covered with Cd, C, and O elements, further revealing that there was a certain quantity of Cd element on the surface of TZ5 cells (Fig. 2). These findings emphasized that TZ5 had good tolerance to Cd, and could adsorb and retain Cd on the cell surfaces.

3.2.2. FTIR analysis

In order to provide further insight into the interaction of functional groups of TZ5 and Cd, FTIR spectra of TZ5 before and after Cd^{2+} treated was carried out (Fig. 3). The wide absorption band around 3408.64 cm^{-1} and 3286.66 cm^{-1} were individually attributed to −OH and –HN stretching vibration of polysaccharides and proteins (Dhal et al., 2010). The peak at 2923.05 cm^{-1} were related to C—H stretching of alkyl group (Karthik et al., 2017). The broad bands observed between 1651.74 cm^{-1} and 1549.11 cm^{-1} was assigned to –NH bending vibration of amide I and amide II bands from peptides and proteins (Wu et al., 2019b). Moreover, there were evident changes in the FTIR spectrum of Cd^{2+} treated TZ5. In summary, the amplitude of most peaks changed, and the wave number of some functional groups increased. These changes indicated TZ5 surface functional groups, such as carboxyl, hydroxyl, alkanes, and amide groups, were contributed to the heavy metal biosorption. Furthermore, heavy metal can modify the cell structure via changing functional groups of the cell surface, which was involved in heavy metal adsorption and bacterial survive in toxic environment (Wu et al., 2019b).

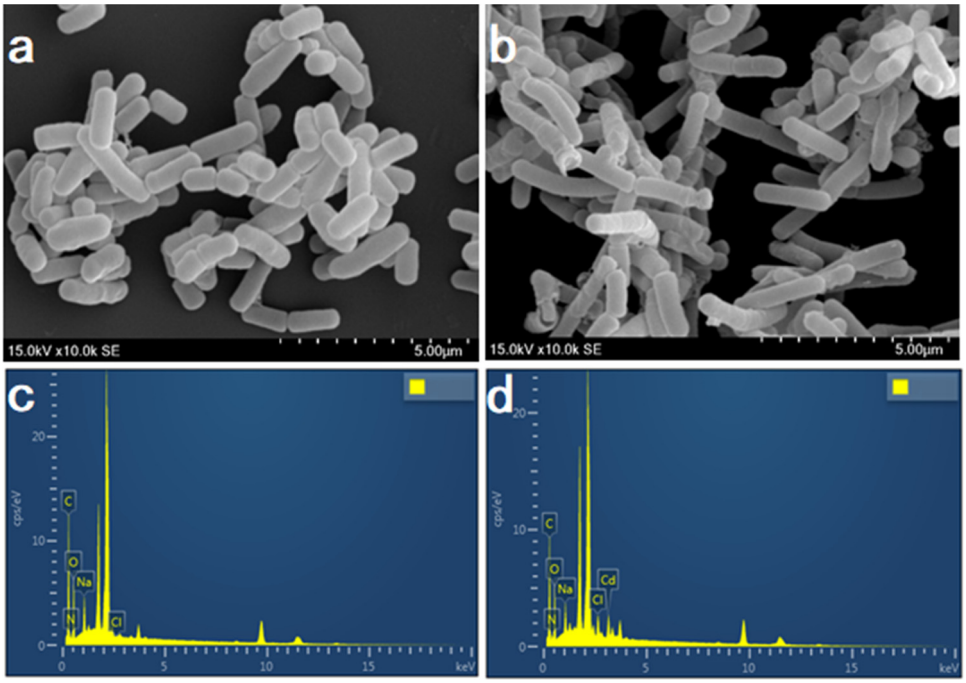

Fig. 1. SEM images of control (a) and Cd^{2+} treated ZT5 cells (b). And EDS analysis of control (c) and Cd^{2+} treated ZT5 cells (d).

3.3. Characterization of BC and BCM

The main physicochemical characteristics of BC was described in Table S5. BC contained high content of organic matter that could support nutrients for growth of TZ5.

Moreover, as illustrated in Fig. 4a, BC exhibited the well-porous structure, further indicating BC was a suitable bacteria loading material. And living cells of strain TZ5 had be successfully immobilized on BC (Fig. 4b), which could provide good habitats for TZ5 colonization and protect TZ5 from direct competition by other bacteria (Quilliam et al., 2013). Besides, FTIR spectra of BC and BCM depicted the possible interaction between bacteria and BC (Fig. 4c). There were plenty of functional groups (such as carboxyl, hydroxyl and amide groups) on the surface of BC (Liu et al., 2018). And the appearance of peaks with increased elongation and intensity in the spectrum of BCM demonstrated that functional groups enriched in the surface of BC were contributed to binding the strain TZ5.

3.4. Fractions distribution of Cd in soil and soil pH

The fractions distribution of Cd in soil was tested and presented in Fig. 5. Compared to CK, the proportion of HOAc-extractable Cd

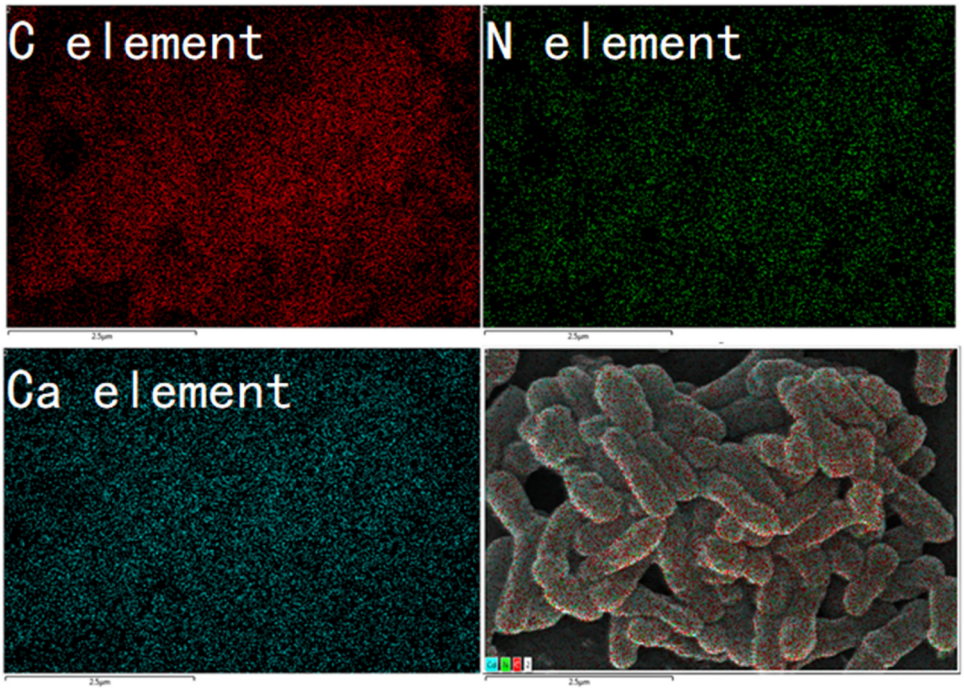

Fig. 2. SEM-EDS images with element mapping of ZT5 in the reaction with Cd^{2+}.

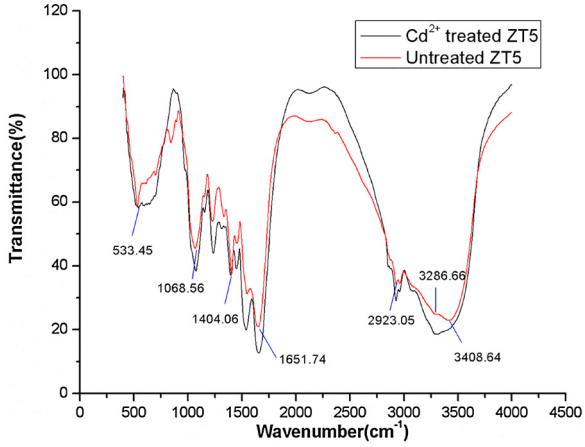

Fig. 3. FTIR spectra of control and Cd^{2+} treated ZT5 cells.

decreased by 4.49 %, 6.05 %, and 11.34 % after incubation with BC, TZ5, and BCM, respectively. And the minimum percentage of HOAc-extractable Cd (36.22 %) was observed in BCM treatments that showed the BCM could effectively immobilize Cd in soil. Meanwhile, compared with CK, the other three fractions (reducible, oxidizable and residual Cd) slightly increased in BC, TZ5 and BCM treatments. The results suggested that the increase of reducible, oxidizable and residual Cd were due to the transformation of HOAc-extractable Cd (Wu et al., 2019c). The variation of soil pH after incubation with amendments were depicted in Fig. S1, and pH values increased by 0.25-0.67 than CK in treatments amended by BC, TZ5 and BCM.

Biochar could increase soil pH, and pH was negatively correlated with the concentration of HOAc-extractable Cd (Liu et al., 2018). Moreover, the functional groups of BC (such as carboxylic and phenolic groups) could interact with heavy metals, and complexes that formed by the interaction between BC and heavy metals were more stable than other complexes that formed by heavy metals and other organic matters in soil (Wu et al., 2019d). For TZ5, it could also increase soil pH. What's more, it was proved that TZ5 could absorb Cd via plenty of active functional groups in this experiments. In addition, TZ5 had good phosphate solubilizing capacity, and soluble phosphorus could chelate heavy metals to immobilize heavy metals (Jinhee et al., 2010). In treatments amended with BCM, TZ5 could thrive and proliferate well due to the presence of BC. And the pH values in BCM treatments were higher than BC and TZ5 treatments. Hence, the results confirmed that BCM was most efficient for Cd immobilization.

3.5. Biomass of ryegrass and Cd concentration in ryegrass

The dry weight of ryegrass and Cd concentration in ryegrass were illustrated in Fig. 6, which showed the effect of different materials on growth response and Cd accumulation of ryegrass. Compared to CK, the biomass of ryegrass increased by 0.42–1.96 g in BC, TZ5 and BCM treatments, respectively (Fig. 6a). As illustrated in Fig. 6b, the concentrations of Cd in amendments treatments were 51.51–78.36 % of CK, and the concentration of Cd in BCM treatments were the lowest (5.45 mg/kg) in all treatments.

Some papers reported that BC could not only reduce toxicity of Cd, but also provide nutrients, resulting to the increase of biomass of plants and the decrease of Cd accumulation in plants (Abbas et al., 2017; Rajendran et al., 2019; Zhang et al., 2016). The increase of biomass of ryegrass inTZ5 treatments might be related to the PGP traits of TZ5. It was proved that TZ5 could produce IAA production and dissolve inorganic phosphorus (Table S3). The production of IAA by PGPB would stimulate root growth and increase nutrients uptake for plants in heavy metal polluted soil (Pramanik et al., 2017). While the phosphate solubilizing bacteria could promote ryegrass growth via converting

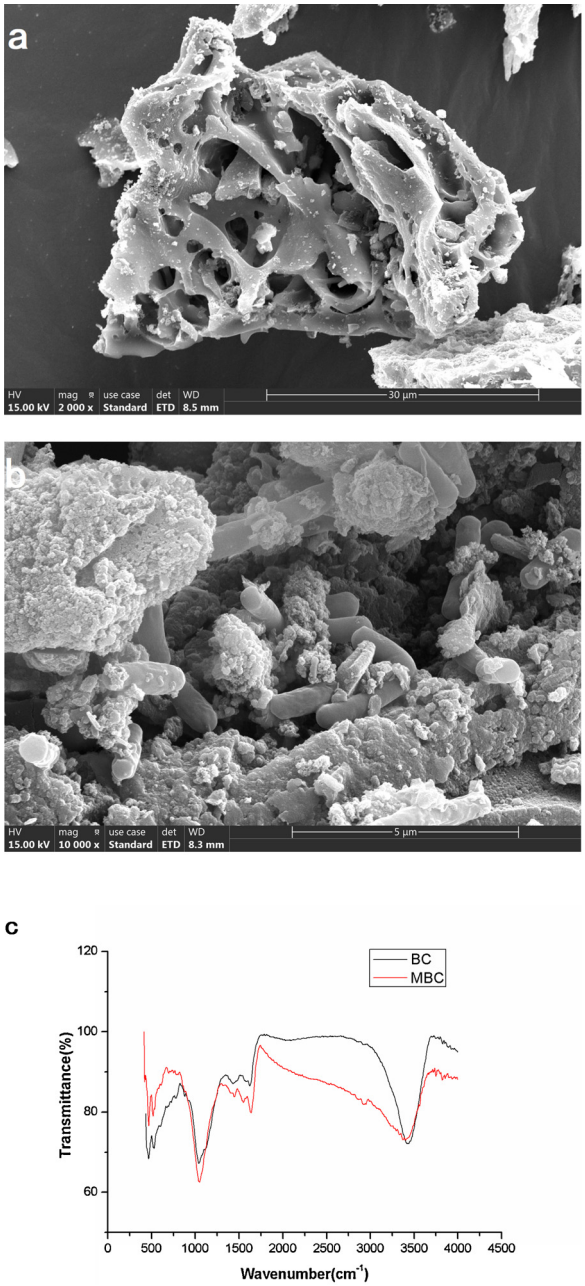

Fig. 4. SEM images of BC (a) and Cd^{2+} treated MBC (b). FTIR spectra of BC and MBC (c).

inorganic phosphate to available phosphate (Rajkumar and Freitas, 2008). In BCM treatments, BC provided shelter and nutrients for TZ5, so TZ5 could secreted more metabolites to promote ryegrass growth. Additionally, the content of HOAc-extractable Cd in soil was lowest in BCM treatments (Fig. 5). Accordingly, BCM could did better in increase biomass of ryegrass and decrease Cd accumulation in ryegrass, compared to BC and TZ5.

3.6. The numbers of microbes and the activities of soil enzymes

To evaluate the bioremediation perform on soil microecological environments of different amendments, the numbers of bacteria and fungi and activities of three important soil enzymes were assayed (Fig. 7). It was conveyed that both the bacterial and fungal counts increased in all treatments. When treated with BC, TZ5 and BCM respectively, the number of bacteria and fungi increased approximately

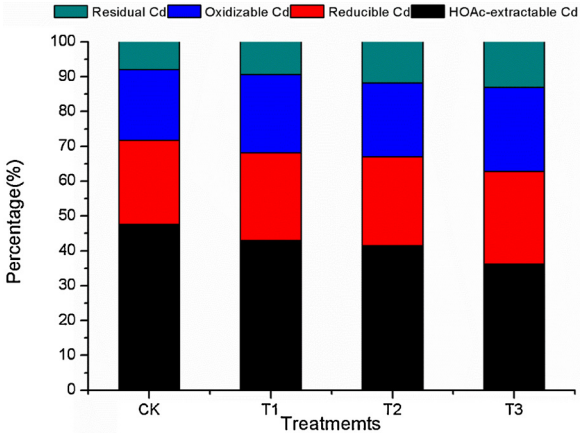

Fig. 5. Percentage of fractions distribution of Cd in soil with different treatments. CK, T1, T2 and T3: amended with 100 mL of 0.85 % sterilized normal saline in soil, amended with 100 mL of BC (5 g) suspension in soil, amended with 100 mL of bacteria suspension in soil and amended with 100 mL of BCM suspension in soil.

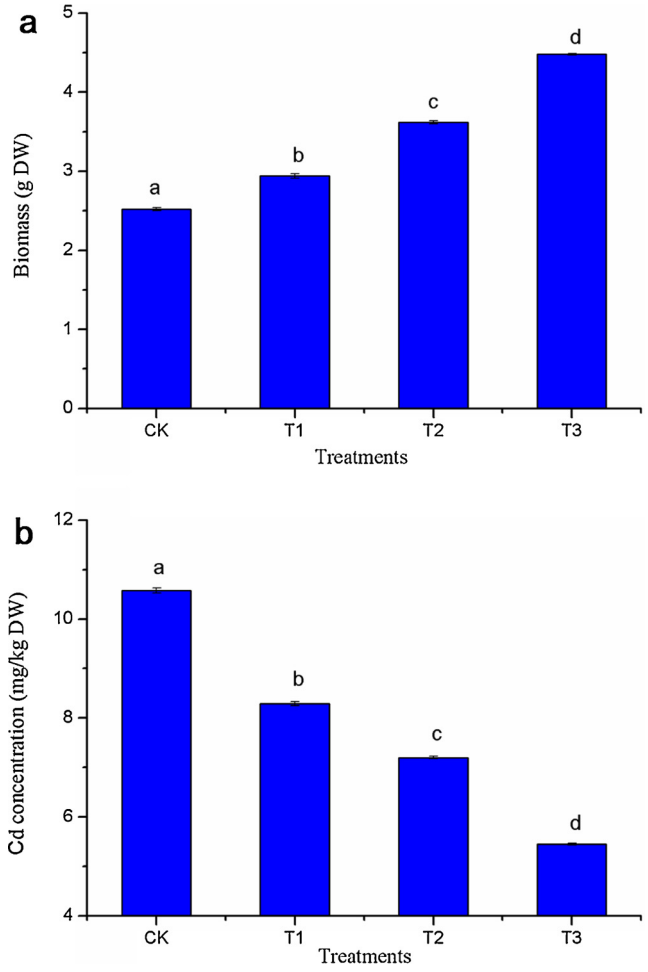

Fig. 6. Biomass of ryegrass (a) and Cd concentration in ryegrass (b) with different treatments. Error bars represented the standard error of three replicates and different letters indicated significant difference ($P \leq 0.05$) among different treatments. CK, T1, T2 and T3: amended with 100 mL of 0.85 % sterilized normal saline in soil, amended with 100 mL of BC (5 g) suspension in soil, amended with 100 mL of bacteria suspension in soil and amended with 100 mL of BCM suspension in soil.

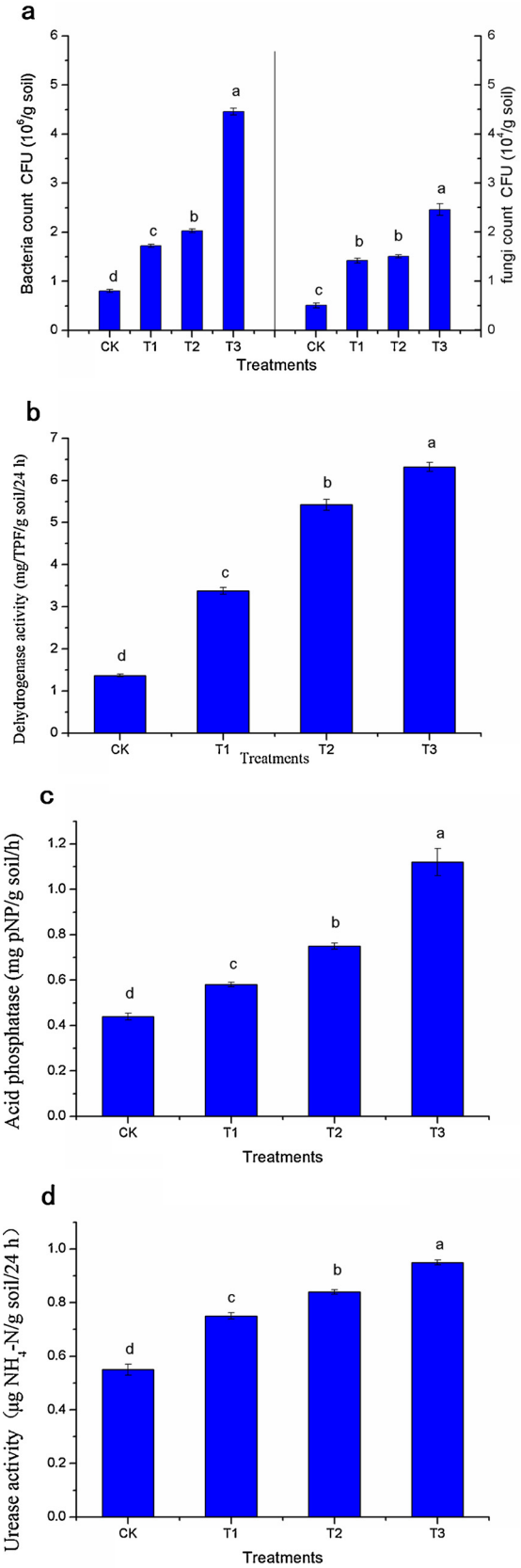

Fig. 7. Microbial counts (a), dehydrogenase activity (b), acid phosphatase activity (c) and urease activity (d) with different treatments. Error bars represented the standard error of three replicates and different letters indicated significant difference ($P \leq 0.05$) among different treatments. CK, T1, T2 and T3: amended with 100 mL of 0.85 % sterilized normal saline in soil, amended with 100 mL of BC (5 g) suspension in soil, amended with 100 mL of bacteria suspension in soil and amended with 100 mL of BCM suspension in soil.

by 1.15–4.58 times and 1.78–3.82 times in comparison to CK (Fig. 7a). Similar increasing trend was presented in soil enzymes activities. As presented in Fig. 7b, the activity of dehydrogenase in BC, TZ5 and BCM treatments was 2.47–4.61 times of CK (Fig. 7c). In BC, TZ5 and BCM treatments, the activity of acid phosphatase increased by 0.32–1.55 % than CK, whereas the activity of urease increased by 0.36–1.73 % than CK (Fig. 7d).

It was reported that heavy metals were hazardous to soil microbes, due to compromise cell membrane integrity, hinder enzyme activity and so on (Poli et al., 2009; Moreno et al., 2003). However, BC and TZ5 could ameliorate Cd toxicity for soil organisms by reducing availability of Cd. Additionally, the metabolites (protein, saccharide, etc.) secreted by interaction of ryegrass and TZ5 would improve the soil microbial activities (Epelde et al., 2009). On the other hand, BC stored plenty of nutrients and could provide shelters for microbes (Wu et al., 2016). Accordingly, the numbers of bacteria and fungi in BCM treatments were the most in all treatments.

The activities of enzymes could further reflect the biochemical qualities of soil microecological environments, and soil enzymes played key roles in the geochemical process of nutrients (Eivazi et al., 2018). The augment of microbial biomass directly resulted in the enhancement of activities of soil enzymes (Li et al., 2019b). Additionally, the active functional groups (carboxyl, hydroxyl, etc.) of BC and TZ5 would chelate Cd to prevent Cd from binding to sulphydryl groups of enzymes (Sanadi, 1982). In summary, the augment of microbial activities caused the enhancement of activities of enzymes. In return, soil enzymes were involved in geochemical cycle of nutrients, resulting in promoting microbes and plants growth. Accordingly, the bioaugmentation of BCM provided a virtuous circle for soil microecological environment in Cd polluted soil.

3.7. Bacterial community structure

In order to further reveal the mechanism of Cd immobilization by BCM, the structure composition of bacterial community was analyzed by high-throughput sequencing. As illustrated in Fig. 8, the relative abundance of bacteria on genus level was significantly influenced by different treatments. And it was observed that the relative abundance of *Bacillus* genus significantly increased in amendments treatments compared to CK. The percentage of relative abundance of *Bacillus* genus in BCM treatments increased by 7.46 % than single bacteria treatments, which sufficiently suggested that it was beneficial for TZ5 colonization using BC as inoculation carriers. It was also proved that *Bacillus* sp. had the ability to reduce Cd accumulation in plants and promote plants growth in previous researches (Wang et al., 2014b; Li et al., 2017), and we got the similar results. Thus, we could conclude the good bioremediation effect in BCM treatments was caused by the large scale proliferation of TZ5.

4. Conclusions

This study indicated that the application of the BCM could effectively increase biomass of ryegrass and decrease Cd accumulation in ryegrass. In addition, the numbers of microbes and activities of soil enzyme were significantly improved in BCM treatments. Moreover, the percentage of relative abundance of *Bacillus* genus in BCM treatments was the most in all treatments, indicating TZ5 was colonized well in soil. In consideration of the low cost, easy accessibility and high efficiency of BCM, the technology was feasible to remediate Cd contaminated sites.

CRediT authorship contribution statement

Hang Ma: Conceptualization, Data curation, Methodology, Software, Writing - original draft, Writing - review & editing. **Mingyang Wei:** Data curation, Software, Writing - original draft. **Ziru Wang:** Data curation, Software. **Siyu Hou:** Visualization. **Xuedan Li:** Methodology. **Heng Xu:** Conceptualization, Funding acquisition, Project administration.

Declaration of Competing Interest

There was no conflict of interest.

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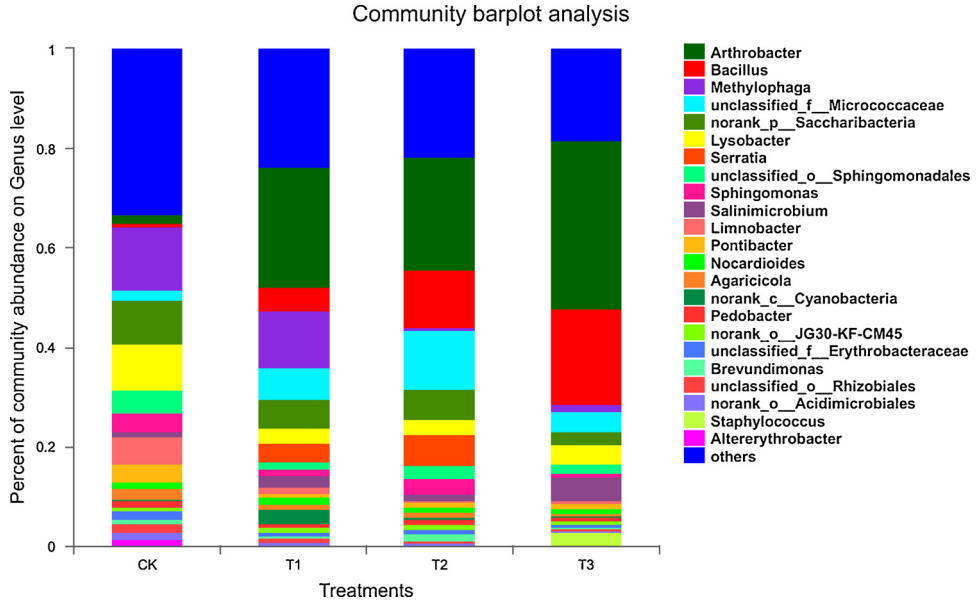

Fig. 8. Percentage of community abundance on genus level. CK, T1, T2 and T3: amended with 100 mL of 0.85 % sterilized normal saline in soil, amended with 100 mL of BC (5 g) suspension in soil, amended with 100 mL of bacteria suspension in soil and amended with 100 mL of BCM suspension in soil.

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Appendix A. Supplementary data

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References

Aarle, I.M.V., Plassard, C., 2010. Spatial distribution of phosphatase activity associated with ectomycorrhizal plants is related to soil type. Soil Biol. Biochem. 42, 324–330.

Abbas, T., Rizwan, M., Ali, S., Zia-Ur-Rehman, M., Qayyum, M.F., Abbas, F., Hannan, F., Rinklebe, J., Yong, S.O., 2017. Effect of biochar on cadmium bioavailability and uptake in wheat (Triticum aestivum L.) grown in a soil with aged contamination. Ecotoxicol. Environ. Saf. 140, 37–47.

Bafana, A., Krishnamurthi, K., Patil, M., Chakrabarti, T., 2010. Heavy metal resistance in Arthrobacter ramosus strain G2 isolated from mercuric salt-contaminated soil. J. Hazard. Mater. 177, 481–486.

Benefield, C.B., Howard, P.J.A., Howard, D.M., 1977. Estimation of dehydrogenase-activity in soil. Soil Biol. Biochem. 9, 67–70.

Bric, J.M., Bostock, R.M., Silverstone, S.E., 1991. Rapid in situ assay for indoleacetic Acid production by bacteria immobilized on a nitrocellulose membrane. Appl. Environ. Microbiol. 57, 535–538.

Byers, H.K., Stackebrandt, E., Hayward, C., Blackall, L.L., 1998. Molecular investigation of a microbial mat associated with the Great Artesian basin. FEMS Microbiol. Ecol. 25, 391–403.

Dhal, B., Thatoi, H., Das, N., Pandey, B.D., 2010. Reduction of hexavalent chromium by Bacillus sp. isolated from chromite mine soils and characterization of reduced product. J. Chem. Technol. Biotechnol. 85, 1471–1479.

Eivazi, F., Afrasiabi, Z., Jose, E., 2018. Effects of silver nanoparticles on the activities of soil enzymes involved in carbon and nutrient cycling. Pedosphere 28, 209–214.

Epelde, L., Mijangos, I., Becerril, J.M., Garbisu, C., 2009. Soil microbial community as bioindicator of the recovery of soil functioning derived from metal phytoextraction with sorghum. Soil Biol Bioch, Soil Biology & Biochemistry 41, 1788–1794.

Fiske, C.H., Subbarow, Y., 1925. The colorimetric determination of phosphorus. J. Biol. Chem. 66, 375–400.

Hale, L., Luth, M., Kenney, R., Crowley, D., 2014. Evaluation of pinewood biochar as a carrier of bacterial strain Enterobacter cloacae UW5 for soil inoculation. Appl. Soil Ecol. 84, 192–199.

Han, Y.H., Fu, J.W., Xiang, P., Cao, Y., Rathinasabapathi, B., Chen, Y., Ma, L.Q., 2017. Arsenic and phosphate rock impacted the abundance and diversity of bacterial arsenic oxidase and reductase genes in rhizosphere of As-hyperaccumulator Pteris vittata. J. Hazard. Mater. 321, 146–153.

Herrmann, L., Lesueur, D., 2013. Challenges of formulation and quality of biofertilizers for successful inoculation. Appl. Microbiol. Biotechnol. 97, 8859–8873.

Huang, F., Dang, Z., Guo, C.L., Lu, G.N., Gu, R.R., Liu, H.J., Zhang, H., 2013. Biosorption of Cd(II) by live and dead cells of Bacillus cereus RC-1 isolated from cadmium-contaminated soil. Colloids Surf. B Biointerfaces 107, 11–18.

Jiang, C., Sun, H., Sun, T., Zhang, Q., Zhang, Y., 2009. Immobilization of cadmium in soils by UV-mutated Bacillus subtilis 38 bioaugmentation and NovoGro amendment. J. Hazard. Mater. 167, 1170–1177.

Jin, H.P., Bolan, N., Megharaj, M., Naidu, R., 2011. Isolation of phosphate solubilizing bacteria and their potential for lead immobilization in soil. J. Hazard. Mater. 185, 829–836.

Jinhee, P., Bolan, N., Megharaj, M., Naidu, R., Kosaki, T., 2010. Isolation of phosphate-solubilizing bacteria and characterization of their effects on lead immobilization. Pedologist.

Kartal, Ş., Aydın, Z., Tokalıoğlu, Ş., 2006. Fractionation of metals in street sediment samples by using the BCR sequential extraction procedure and multivariate statistical elucidation of the data. J. Hazard. Mater. 132, 80–89.

Karthik, C., Barathi, S., Pugazhendhi, A., Ramkumar, V.S., Ngoc Bao Dung, T., Arulselvi, P.I., 2017. Evaluation of Cr(VI) reduction mechanism and removal by Cellulosimicrobium funkei strain AR8, a novel haloalkaliphilic bacterium. J. Hazard. Mater. 333, 42–53.

Khan, F.I., Husain, T., Hejazi, R., 2004. An overview and analysis of site remediation technologies. J. Environ. Manage. 71, 95–122.

Li, L., Wang, S., Li, X., Li, T., He, X., Tao, Y., 2018. Effects of Pseudomonas chenduensis and biochar on cadmium availability and microbial community in the paddy soil. Sci. Total Environ. 640, 1034–1043.

Li, X., Dong, S., Yao, Y., Shi, W., Wu, M., Xu, H., 2016. Inoculation of bacteria for the bioremediation of heavy metals contaminated soil by Agrocybe aegerita. RSC Adv. 6, 65816–65824.

Li, Y., Pang, H.D., He, L.Y., Wang, Q., Sheng, X.F., 2017. Cd immobilization and reduced tissue Cd accumulation of rice (Oryza sativa wuyun-23) in the presence of heavy metal-resistant bacteria. Ecotoxicol. Environ. Saf. 138, 56–63.

Li, X., Ma, H., Li, L., Gao, Y., Li, Y., Xu, H., 2019a. Subcellular distribution, chemical forms and physiological responses involved in cadmium tolerance and detoxification in Agrocybe Aegerita. Ecotoxicol. Environ. Saf. 171, 66–74.

Li, X., Xiao, K., Ma, H., Li, L., Tan, H., Xu, H., Li, Y., 2019b. Mechanisms into the removal and translocation of cadmium by Oudemansiella radicata in soil. Environ. Sci. Pollut. Res. - Int. 26, 6388–6398.

Liu, Y., Boqing, T., Yuanxinglu, L., Ming, L., Xiangdong, W., Xiaoli, L., Huihui, D., 2020. Inoculation of soil with cadmium-resistant bacterium Delftia sp. B9 reduces cadmium accumulation in rice (Oryza sativa L.) grains. Ecotoxicol. Environ. Saf.

Liu, H., Xu, F., Xie, Y., Wang, C., Zhang, A., Li, L., Xu, H., 2018. Effect of modified coconut shell biochar on availability of heavy metals and biochemical characteristics of soil in multiple heavy metals contaminated soil. Sci. Total Environ. 645, 702–709.

Liu, H., Guo, S., Jiao, K., Hou, J., Xie, H., Xu, H., 2015. Bioremediation of soils co-contaminated with heavy metals and 2,4,5-trichlorophenol by fruiting body of Clitocybe maxima. J. Hazard. Mater. 294, 121–127.

Ma, Y., Rajkumar, M., Zhang, C., Freitas, H., 2016. Inoculation of Brassica oxyrrhina with plant growth promoting bacteria for the improvement of heavy metal phytoremediation under drought conditions. J. Hazard. Mater. 320, 36–44.

Masiello, C.A., Chen, Y., Gao, X., Liu, S., Cheng, H.-Y., Bennett, M.R., Rudgers, J.A., Wagner, D.S., Zygourakis, K., Silberg, J.J., 2013. Biochar and microbial signaling: production conditions determine effects on microbial communication. Environ. Sci. Technol. 47, 11496–11503.

Meharg, A.A., Gareth, N., Claire, D., Paul, W., Adomako, E.E., Adam, P., Yongguan, Z., Gang, L., Fang-Jie, Z., Steve, M.G., 2013. Variation in rice cadmium related to human exposure. Environ. Sci. Technol. 47, 5613–5618.

Mitra, S., Pramanik, K., Sarkar, A., Ghosh, P.K., Soren, T., Maiti, T.K., 2018. Bioaccumulation of cadmium by Enterobacter sp. and enhancement of rice seedling growth under cadmium stress. Ecotoxicol. Environ. Saf. 156, 183–196.

Moreno, J.L., Garcia, C., Hernandez, T., 2003. Toxic effect of cadmium and nickel on soil enzymes and the influence of adding sewage sludge. Eur. J. Soil Sci. 54, 377–386.

Poli, A., Salerno, A., Laezza, G., Donato, P.D., Dumontet, S., Nicolaus, B., 2009. Heavy metal resistance of some thermophiles: potential use of α-amylase from Anoxybacillus amylolyticus as a microbial enzymatic bioassay. Res. Microbiol. 160, 99–106.

Pramanik, K., Mitra, S., Sarkar, A., Maiti, T.K., 2018. Alleviation of phytotoxic effects of cadmium on rice seedlings by cadmium resistant PGPR strain Enterobacter aerogenes MCC 3092. J. Hazard. Mater. 351, 317–329.

Pramanik, K., Mitra, S., Sarkar, A., Soren, T., Maiti, T.K., 2017. Characterization of cadmium-resistant Klebsiella pneumoniae MCC 3091 promoted rice seedling growth by alleviating phytotoxicity of cadmium. Environ. Sci. Pollut. Res. - Int. 24, 24419–24437.

Prapagdee, B., Chanprasert, M., Mongkolsuk, S., 2013. Bioaugmentation with cadmium-resistant plant growth-promoting rhizobacteria to assist cadmium phytoextraction by Helianthus annuus. Chemosphere 92, 659–666.

Pueyo, M., Lopez-Sanchez, J.F., Rauret, G., 2004. Assessment of CaCl 2, NaNO 3 and NH 4 NO 3 extraction procedures for the study of Cd, Cu, Pb and Zn extractability in contaminated soils. Anal. Chim. Acta 504, 217–226.

Quilliam, R.S., Glanville, H.C., Wade, S.C., Jones, D.L., 2013. Life in the 'charosphere' – does biochar in agricultural soil provide a significant habitat for microorganisms? Soil Biol. Biochem. 65, 287–293.

Rajendran, M., Shi, L., Wu, C., Li, W., An, W., Liu, Z., Xue, S., 2019. Effect of sulfur and sulfur-iron modified biochar on cadmium availability and transfer in the soil-rice system. Chemosphere 222, 314–322.

Rajkumar, M., Freitas, H., 2008. Influence of metal resistant-plant growth-promoting bacteria on the growth of Ricinus communis in soil contaminated with heavy metals. Chemosphere 71, 834–842.

Reddy, K.R., Chinthamreddy, S., Saichek, R.E., Cutright, T.J., 2003. Nutrient amendment for the bioremediation of a chromium-contaminated soil by electrokinetics. Energy Sources 25, 931–943.

Sanadi, D.R., 1982. Mitochondrial coupling Factor-B - properties and role in ATP synthesis. Biochim. Biophys. Acta 683, 39–56.

Wang, T., Sun, H., Mao, H., Zhang, Y., Wang, C., Zhang, Z., Wang, B., Sun, L., 2014a. The immobilization of heavy metals in soil by bioaugmentation of a UV-mutant Bacillus subtilis 38 assisted by NovoGro biostimulation and changes of soil microbial community. J. Hazard. Mater. 278, 483–490.

Wang, T., Sun, H., Jiang, C., Mao, H., Zhang, Y., 2014b. Immobilization of Cd in soil and changes of soil microbial community by bioaugmentation of UV-mutated Bacillus subtilis 38 assisted by biostimulation. Eur. J. Soil Biol. 65, 62–69.

Wu, H., Zeng, G., Liang, J., Chen, J., Xu, J., Dai, J., Li, X., Chen, M., Xu, P., Zhou, Y., 2020. Responses of bacterial community and functional marker genes of nitrogen cycling to biochar, compost and combined amendments in soil. Appl. Microbiol. Biotechnol. 100, 8583–8591.

Wu, H., Lai, C., Zeng, G., Liang, J., Wan, J., 2016. The interactions of composting and biochar and their implications for soil amendment and pollution remediation: a review. Crit. Rev. Biotechnol. 37, 1–11.

Wu, H., Chen, J., Xu, J., Zeng, G., Sang, L., Liu, Q., Yin, Z., Dai, J., Yin, D., Lang, J., Ye, S., 2019a. Effects of dam construction on biodiversity: a review. J. Clean. Prod. 221, 480–489.

Wu, M., Li, Y., Li, J., Wang, Y., Xu, H., Zhao, Y., 2019b. Bioreduction of hexavalent chromium using a novel strain CRB-7 immobilized on multiple materials. J. Hazard. Mater. 368, 412–420.

Wu, B., Wang, Z., Zhao, Y., Gu, Y., Wang, Y., Yu, J., Xu, H., 2019c. The performance of biochar-microbe multiple biochemical material on bioremediation and soil microecology in the cadmium aged soil. Sci. Total Environ. 686, 719–728.

Wu, C., Shi, L., Xue, S., Li, W., Jiang, X., Rajendran, M., Qian, Z., 2019d. Effect of sulfur-iron modified biochar on the available cadmium and bacterial community structure in contaminated soils. Sci. Total Environ. 647, 1158–1168.

Xiao, K., Li, Y., Sun, Y., Liu, R., Li, J., Zhao, Y., Xu, H., 2017. Remediation performance and mechanism of heavy metals by a bottom up activation and extraction system using multiple biochemical materials. ACS Appl. Mater. Interfaces 9, 30448–30457.

Yan, J., Quan, G., Cheng, D., 2013. Effects of the combined pollution of lead and cadmium on soil urease activity and nitrification⋆. Procedia Environ. Sci. 18, 78–83.
Ye, S., Zeng, G., Wu, H., Zhang, C., Dai, J., Liang, J., Yu, J., Ren, X., Yi, H., Cheng, M., 2020. Biological technologies for the remediation of co-contaminated soil. Crit. Rev. Biotechnol. 1–15.
Ye, S., Zeng, G., Wu, H., Liang, J., Zhang, C., Dai, J., Xiong, W., Song, B., Wu, S., Yu, J., 2019. The effects of activated biochar addition on remediation efficiency of co-composting with contaminated wetland soil. Resour. Conserv. Recycl. 140, 278–285.
Zeng, P., Guo, Z.H., Xiao, X.Y., Peng, C., Feng, W.L., Xin, L.Q., Xu, Z., 2019. Phytoextraction potential of Pteris vittata L. co-planted with woody species for As, Cd, Pb and Zn in contaminated soil. Sci. Total Environ. 650, 594–603.
Zeng, G., Wu, H., Liang, J., Guo, S., Huang, L., Xu, P., Liu, Y., Yuan, Y., He, X., He, Y., 2015. Efficiency of biochar and compost (or composting) combined amendments for reducing Cd, Cu, Zn and Pb bioavailability, mobility and ecological risk in wetland soil. RSC Adv. 5, 34541–34548.
Zhang, G., Guo, X., Zhao, Z., He, Q., Wang, S., Zhu, Y., Yan, Y., Liu, X., Sun, K., Zhao, Y., Qian, T., 2016. Effects of biochars on the availability of heavy metals to ryegrass in an alkaline contaminated soil. Environ. Pollut. 218, 513–522.