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# Effect of cornstalk biochar on phytoremediation of Cd-contaminated soil by *Beta vulgaris* var. *cicla* L

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ABSTRACT

Cadmium (Cd) contamination is the most common and extensive heavy metal pollution in the farmland of China. Phytoremediation is considered as a promising measure for Cd-contaminated soil remediation, but the remediation efficiency still needs to be enhanced. Biochar as an effective amendment medium is widely manufactured and studied for the soil remediation of heavy metals. In this study, a greenhouse pot trial was conducted to investigate the effects of cornstalk biochar on Cd accumulation of *Beta vulgaris* var. *cicla* L. (*Beta vulgaris*) in Cd contaminated soil. The Cd availability, speciation and nutrients in soil, biomass and Cd chemical forms in the *Beta vulgaris* root were studied to explore the mechanism that how the cornstalk biochar promoted Cd accumulation in *Beta vulgaris*. Biochar amendment reduced the DTPA-extractable Cd concentration and stimulated the growth of root. Compared to the *Beta vulgaris* without biochar treatment, the results of 5% biochar amendment showed that the root dry weight of *Beta vulgaris* increased to 267%, Cd accumulation in *Beta vulgaris* increased to 206% and the Cd concentration in leaves and roots increased by 36% and 52%, respectively. Additionally, after 5% biochar was applied to soil, the total content of organic matter-bound Cd and residual Cd increased by 38%, while the content of Fe–Mn oxides-bound Cd decreased by 40%. Meanwhile, Cd may mainly bind to the root cell wall and the ratio of NaCl-extracted Cd to HAc-extracted Cd increased to 166% with 5% biochar amendment. According to our study, Cd in soil can be removed by *Beta vulgaris* and phytoremediation efficiency can be improved with biochar amendment. The combination of phytoremediation and biochar amendment is a promising strategy for the Cd-contaminated soil remediation.

## 1. Introduction

Cd is a highly toxic and mobile heavy metal with great bioaccumulation and biomagnification potential that can be transferred to the food chain, posing a significant threat to food security and human health (de Godoi Pereira et al., 2018; Hamid et al., 2020; He et al., 2017). Furthermore, the area of Cd pollution in farmland reached about $2.786 \times 10^9$ $m^2$ in China (Liang et al., 2018). Consequently, it is a pressing matter of the moment to remediate Cd-contaminated soil. Biochar as a resultful amendment medium and used widely for heavy metal immobilization in recent years (Nejad et al., 2018). It could quickly and effectively reduce the Cd mobility in soils and the heavy metal concentration in crops (Bian et al., 2014), such as rice, wheat, etc. However, the heavy metals immobilized by biochar have the risk of re-release (Hou et al., 2016). Phytoremediation is an economical and environmentally friendly remediation approach and considered as a promising measure for in-situ Cd-contaminated soil remediation (Sarwar et al., 2017). Nevertheless, the long remediation period limites the practical application of phytoremediation (Paz-Ferreiro et al., 2014). If biochar could immobilize heavy metals quickly and has no remarkable inhibitory effect on phytoremediation, then heavy metal contaminated farmland could be farmed after remediation process by the combined strategy of phytoremediation and biochar amendment.

Some studies have indicated that biochar didn't inhibit and even

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enhanced the uptake and accumulation of heavy metals by plants although the bioavailability of heavy metals in soils and sediments was reduced by biochar (Hartley et al., 2009; Lebrun et al., 2017). Many hyperaccumulators assisted with biochar could exhibit the above features. Rees et al. (2015) reported that 5% coniferous and hardwood chips biochar amendment promoted Zn and Cd uptake in the hyperaccumulator *Noccaea caerulescens*. However, the biochar reduced bioavailable Zn and Cd content in soils extracted with DTPA and the content in non-hyperaccumulator *Lolium perenne*, both in acidic and alkaline soils. In another study of Rees et al. (2016), similar results were found. Adding 5% hardwood and softwood biochar reduced the Cd–Zn content in *Zea mays* L., but it did not affect Cd–Zn uptake of the hyperaccumulator *N.caerulescens* in alkaline soil. Li et al. (2018a) found the addition of 5% straw biochar increased Cd concentration in hyperaccumulator *Sedum plumbizincicola*, while it decreased soil available Cd by 60% in a spiked and acidic agricultural soil.

The enhancement of Cd accumulation in plants was attributed to the fact that biochar increased nutrients in soils (such as P, N and C) and promoted the root development of plants (Rees et al., 2016). Jain et al. (2016) found that available phosphorus (AP) and soil organic carbon were significantly improved by lemongrass biochar. Rees et al. (2016) found that the 5% wood biochar induced root proliferation and increased root surface of *L.perenne* and *N.caerulescens* in alkaline soil. The content of Zn and Cd in hyperaccumulator *N.caerulescens* increased, while the content of Zn and Cd in non-accumulator *L. perenne* decreased. The straw biochar increased Cd uptake of hyperaccumulator *S. plumbizincicola* in a spiked and acidic agricultural soil by promoting the root morphology of the plant (Li et al., 2018a). The root morphological development makes the soil range of heavy metals that could be absorbed by plant more reasonable, but whether it promotes the uptake of heavy metals depends on the plant species. In addition, even heavy metal speciation characteristics in soils were speculated to be affected by increased soil nutrients, with all of these changes impairing the absorption of heavy metals by plants (Aller, 2016; Paz-Ferreiro et al., 2014).

However, the increase of soil nutrition and root growth are insufficient to account for enhancement of heavy metals accumulation in plants by biochar. The accumulation of heavy metals in plants depended not only on plant biomass, but also on the concentration of heavy metals in plants (Li et al., 2018b). Factors such as the plant species, heavy metal speciation, and soil properties, etc. could also interfere with heavy metal uptake by plants (Paz-Ferreiro et al., 2014). Consequently, it is necessary to explore the relationships among plant growth, the accumulation of heavy metal in plants, the changes of soil nutrition and heavy metal speciation characteristics after biochar amendment.

The cell wall of plant roots is the first structure in contact with extracellular Cd, meanwhile, it is also an effective barrier to Cd uptake. Wu et al. (2005) found greater NaCl-extractable Cd fraction in tolerant than in sensitive barley accessions by comparing Cd chemical forms in Cd-sensitive and Cd-tolerant barley. Cd bound to protein and pectin (NaCl-extracted Cd) accounted for the highest proportion in hyperaccumulator *Phytolacca americana* L. as Fu et al. (2011) have studied. Pectin molecules account for 30% of the primary cell wall, yet they contain up to 70% of the negatively charged groups, which are considered to be critical molecules for isolating divalent ions (Loix et al., 2017). In addition, a high proportion of pectin and protein integrated ions is considered to be adsorbed on the cell walls (Zhang et al., 2019). Thus, investigating for Cd chemical forms of plant roots to explore the influence of biochar on Cd accumulation in plants is indispensable.

Hyperaccumulators of Cd not only grow slowly and have small biomass, but also are sensitive to environment (Sarwar et al., 2017). *Beta vulgaris* var. *cicla* L. (*Beta vulgaris*) is a superior alternative plant as Cd hyperaccumulator candidate. *Beta vulgaris* has large biomass, short growing period and high Cd extraction capacity (Chen et al., 2013; Song et al., 2012; Yushuang et al., 2007).

The purpose of this study was to investigate whether the accumulation of Cd in *Beta vulgaris* would be inhibited with different proportion of cornstalk biochar amendment in Cd contaminated soil. The changes of soil nutrients available nitrogen (AN), AP, labile organic carbon (LOC), atomic ratio of C/N, etc.), Cd availability and speciation characteristics in soils, plant biomass, and Cd chemical forms in roots were investigated for the influences of biochar amendment on Cd accumulation in *Beta vulgaris*. This study could provide a reference for the combined strategy of phytoremediation and biochar amendment for remediation of Cd-contaminated soil.

## 2. Materials and methods

### 2.1. Soil and biochar

The soil used in the experiment was collected from the surface soil (0–25 cm) of farmland in the eastern campus of Shanghai University, China. Before spiked, air-dried soil was ground and passed through nylon mesh sieve (2-mm) (Hu et al., 2017). The Cd-contaminated soil was prepared by adding the aqueous solution of $Cd(NO_3)_2$ and aging for two months before the pot experiment (Wei et al., 2014). The basic properties of the contaminated soil are listed in Table 1.

The feedstock of biochar was cornstalk. Broken cornstalk was placed in a specialized, sealed iron can and pyrolyzed under $N_2$-condition (4 °C $min^{-1}$ to 500 °C) for 4 h (Wang et al., 2013a). The biochar was passed through 0.15 mm sieve before use. The elemental analyses of C, H and N of biochar were analyzed with an elemental analyzer (EA3000, Dalton International, Italy). The determination method of Ca, Mg, etc. of biochar referred to Du et al. (2019). The basic properties of the biochar are listed in Table 2.

### 2.2. Pot experiment

The pot experiment was conducted in an artificial atmospheric phenomena simulator (East campus, Shanghai University). 1.0 kg dry soil was added in every plastic pot (height of 12.5 cm, bottom and top diameter of 15 cm and 18 cm, respectively). Six treatment groups were designed: *Beta vulgaris* without biochar, no *Beta vulgaris* without biochar, *Beta vulgaris* with 1% biochar, no *Beta vulgaris* with 1% biochar, *Beta vulgaris* with 5% biochar, no *Beta vulgaris* with 5% biochar. The different proportion of biochar (w/w) were added into soil and mixed well. *Beta vulgaris* seeds germinated on three-layer filter paper beds within 10 days. And then six seedlings were transplanted in each pot evenly (Chen et al., 2013; Mogren et al., 2016). Every treatment was performed in triplicate. During the experiment, 60% of water holding capacity was kept. All the plants were harvested after 60 days' growth from the date of seedlings transplanting.

### 2.3. Sample collection

After harvesting, the roots, stems and leaves of the plants were separated with hand and weighed. The stems and leaves of the plants

**Table 1**
Physicochemical properties of the soil before the pot experiment.

| Properties | Value |
|---|---|
| pH | 7.90 ± 0.12 |
| Cd (mg $kg^{-1}$) | 3.73 ± 0.16 |
| Total organic carbon (mg $g^{-1}$) | 31.25 ± 0.23 |
| Total nitrogen (mg $g^{-1}$) | 3.13 ± 0.15 |
| Available nitrogen (mg $kg^{-1}$) | 96.60 ± 2.97 |
| Available phosphorus (mg $kg^{-1}$) | 14.29 ± 0.20 |
| Available potassium (mg $kg^{-1}$) | 19.80 ± 0.18 |
| Clay (%) | 5.02 |
| Silt (%) | 51.03 |
| Sand (%) | 43.95 |
| Agrotype | silty loam |

**Table 2**
Physicochemical properties of the cornstalk biochar before the pot experiment.

| Properties | Value |
|---|---|
| pH (biochar: water = 1: 10, w/v) | 10.49 ± 0.02 |
| Ash (%) | 11.51 ± 0.16 |
| C (mg $g^{-1}$) | 778.38 ± 2.39 |
| O (mg $g^{-1}$) | 155.90 ± 1.21 |
| N (mg $g^{-1}$) | 23.08 ± 0.03 |
| H (mg $g^{-1}$) | 33.34 ± 0.16 |
| Cd (mg $kg^{-1}$) | Not detected |

were freeze-dried and ground for subsequent analysis. The roots were divided into two subsamples. The first set of subsamples was processed like the stems and leaves. The other subsamples were placed in 4 °C refrigerator for morphological analysis of Cd in plant roots (Fu et al., 2011). Soil samples of all treatment groups were mixed separately and thoroughly, then divided into two subsamples. One was placed in a −80 °C refrigerator for soil microbial activity analysis and the other subsample was freeze-dried and sieved through 2 mm for further analysis.

### 2.4. Soil nutrients

The concentrations of soil AN (alkalized nitrogen method) and AP (0.5 M $NaHCO_3$ extraction) were determined as described by Lu (1999). Briefly, for AN analysis, 2.0 soil sample was evenly spread in the outer chamber of the diffusion dish with 2 mL 2% boric acid solution and 1 drop of mixed indicator. Then applied alkaline glycerin on the edge of the outer chamber and covered the glass sheet. Turned the glass cover to expose one side of the outer chamber at the notch slowly, added 10 mL 1.0 M NaOH solution and immediately covered tightly, carefully rotated the diffusion dish to mix well. After incubation for 24 h at 40°C, then titrated with 0.01 M $H_2SO_4$ standard solution. For AP analysis, 2.5 g soil sample was accurately weighed into a 150 mL triangle bottle with 50 mL 0.5 M $NaHCO_3$ and shaken for 30 min (25°C). Then 10 mL filtrate was taken into a 50 mL capacity bottle and 5 mL molybdenum antimony anti-mixed chromogenic agent was added along the bottle wall slowly. The mixture was shaken and the absorbance of the samples were measured at 485 nm after 30 min. The LOC content was determined using the potassium permanganate oxidation method (Wang et al., 2005). The total organic carbon (TOC) and total nitrogen (TN) content were measured using an elemental analyzer (Wang et al., 2013b).

### 2.5. Available Cd and the fractions in soil

The influences of biochar and plants on Cd mobility in soils were investigated with $CaCl_2$ extraction method. The Cd concentration extracted with 0.01 M $CaCl_2$ was strongly corresponded with the level in the soil solution (Meers et al., 2007a) and proved to be the most versatile procedure as it provided a good indication of phytoavailability under evaluation (Meers et al., 2007b; Xu et al., 2018). Briefly, 5.0 g of air-dried soil was added into 25 mL 0.01 M $CaCl_2$ solution in a flask and the mixture was shaken at 200 r/min for 2 h. Then the suspension was centrifuged at 3000 r/min for 10 min and filtered through 0.45 μm mixed cellulose esters filters. The concentration of $CaCl_2$-extracted Cd was analyzed by graphite furnace-atomic absorption spectrometry (GF-AAS ZEEnit 600, Analytik Jena, Germany).

The effect of biochar and plants on Cd phytoavailability in soils was also investigated with DTPA (pH 7.3, 0.1 M TEA, 0.01 M $CaCl_2$, 0.005 M DTPA). 10 g of air-dried soil was immersed into 20 mL DTPA solution and the mixture was shaken at 200 rmp for 2 h. After centrifugation (3000 r/min, 10 min), the suspension was filtered through slow speed quantitative filter paper for testing. The concentration of DTPA-extracted Cd was analyzed by GF-AAS.

The Cd fractions in soils were according to the sequential extraction (Tessier et al., 1979). Exchangeable, bound to carbonates, bound to Fe–Mn oxides, bound to organic matter and residual Cd were included. The determination of Cd concentration in different extraction solutions was by GF-AAS.

### 2.6. Concentration of Cd in plants

Leaf, stem and root (0.2 g of dry weight) were ground and mixed, respectively. The samples were digested with mixed acid of 5 mL $HNO_3$ and 3 mL $HClO_4$ in a polytetrafluoroethylene tube to be completely dissolved without precipitation (200°C for about 2 h). The digestion solution was diluted with 0.5% $HNO_3$ to 25 mL and determined by GF-AAS.

### 2.7. Concentration of Cd chemical forms in roots

The Cd chemical forms determination of *Beta vulgaris* in roots referred to Fu et al. (2011). Fresh root samples were sequentially extracted with 80% ethanol (mainly extract inorganic Cd giving priority to aminophenol, chloride, and nitrate/nitrite), deionized water (extract $Cd_2PO_4$, and water-soluble Cd of organic acid), 1 M NaCl (extract Cd integrated with protein and pectates), 2% acetic acid (HAc) (extract undissolved Cd-phosphate, such as $Cd_3(PO_4)_2$ and $CdHPO_4$), 0.6 M HCl (extract Cd-oxalic acid), respectively, the last sediment was residual fraction and then digested with mixed acid $HClO_4$: $HNO_3$ (13:87, v/v). Simply, fresh root samples (1.0 g) were added into 20 mL extractant and ground to homogenate (4 °C). Then transferred the homogenate to a 50 mL plastic centrifuge tube. The mixture was centrifuged and poured out the first supernatant. The sedimentation was re-suspended twice with 10 mL the same extractant. The mixture was shaken and centrifuged, and then the supernatant was poured out, respectively. Finally, three supernatants were mixed. Each of the combined supernatants was evaporated to constant weight on a plate (70 °C) and digested with mixed acid of 5 mL $HNO_3$ and 3 mL $HClO_4$. All of Cd concentrations in the solution were analyzed using GF-AAS.

### 2.8. Statistical analysis

All the data were processed with Microsoft Excel and SPSS 17.0 using $p < 0.05$ to test differences unless otherwise stated. The standard deviation (SD) obtained from the three parallel samples was shown as error bars in the figures. Data analysis was performed using one-way ANOVA (Duncan's multiple range tests) with a 95% confidence level. Spearman correlation analysis was conducted using SPSS 17.0 to investigate the correlations among root biomass, Cd content and soil nutrients.

## 3. Results

### 3.1. Cd accumulation and uptake in Beta vulgaris

Cd concentrations in leaf, shoot and root of *Beta vulgaris* with different doses of cornstalk biochar treatment are presented in Fig. 1. Comparing with the treatment without biochar, there was no significant influence on the Cd accumulation in *Beta vulgaris* with the addition of 1% biochar. Cd accumulation in *Beta vulgaris* increased to 206% with 5% biochar amendment. In the terms of Cd concentrations in leaf, stem and root of *Beta vulgaris*, the addition of 1% biochar has no effect on the Cd uptake in *Beta vulgaris* compared with the treatment without biochar. The Cd concentration in leaves and roots of *Beta vulgaris* with the addition of 5% biochar increased by 36% and 52% compared with no-biochar treatment (from 10.52 to 25.35 μg $g^{-1}$ to 17.89 and 38.46 μg $g^{-1}$), respectively. No significant difference was obtained in the Cd concentration of *Beta vulgaris* stem among different treatment groups.

### 3.2. Available Cd in soil

The biochar could stabilize heavy metals and reduce the available Cd

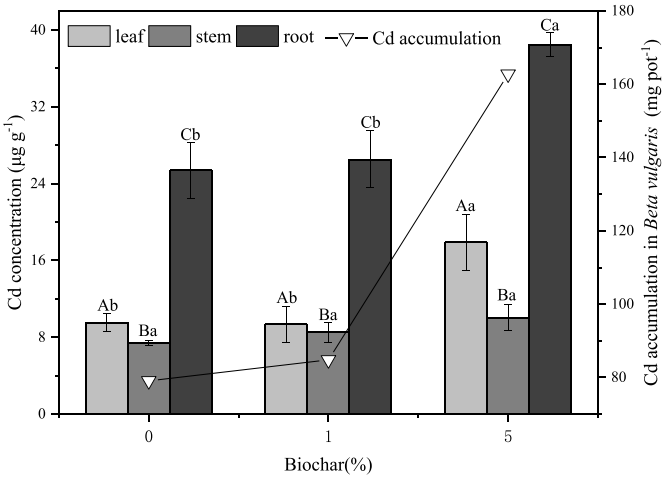


**Fig. 1.** Cd concentrations in leaf, stem and root of Beta vulgaris and Cd accumulation of Beta vulgaris in per pot with different doses of cornstalk biochar amendment. 0, 1 and 5 meant the application at doses of 0, 1 and 5% (w/w) cornstalk biochar, respectively. (Data are mean ± SD, n = 3. Error bars denote the standard deviations. Different small letters with the same capital letter indicate statistically significant differences among groups ($P < 0.05$)).

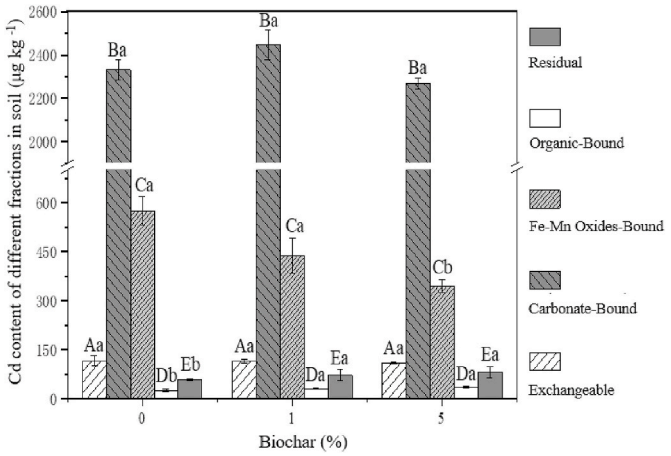


**Fig. 3.** Different chemical fractions of Cd in soil with different treatment. 0, 1 and 5 meant the application at doses of 0, 1 and 5% (w/w) cornstalk biochar with *Beta vulgaris*, respectively. (Data are mean ± SD, n = 3. Error bars denote the standard deviation. Different small letters with the same capital letter indicate statistically significant differences among groups ($P < 0.05$)).

in soil. The Cd concentrations extracted by 0.01 M $CaCl_2$ with different doses of cornstalk biochar treatment are shown in Fig. 2a. The $CaCl_2$-extracted Cd concentration of the treatment group without *Beta vulgaris* decreased by 10% after 5% biochar amendment. When *Beta vulgaris* were planted, there was no significant difference among treatments. Fig. 2b demonstrated the concentrations of Cd extracted by DTPA with different biochar proportion treatment. In the treatment without *Beta vulgaris*, there was an insignificant decrease of DTPA-extracted Cd with the amendment of 1% biochar compared to without biochar amendment and Cd extracted by DTPA decreased by 29% at the amendment of 5% biochar. In the treatment planted *Beta vulgaris*, the DTPA-extracted Cd concentration in 1% biochar amended soil had no significant difference compared with biochar-free soil. However, the concentration of Cd extracted by DTPA decreased by 16% after 5% biochar amendment.

### 3.3. Fractions of Cd in soil

Different Cd fractions and its concentration in the soil planted *Beta vulgaris* with different doses of biochar treatment are manifested in Fig. 3. There was no significant difference in the exchangeable fraction and carbonate-bound fraction with the dose of biochar increased. Nevertheless, it was noteworthy that compared with the soils planted *Beta vulgaris* which didn't amend with biochar, the Fe–Mn oxides-bound fraction decreased by 24% and 40% with 1% and 5%, respectively, of biochar amendment. The total content of organic matter-bound Cd and residual Cd increased by 22% and 38% with 1% and 5% of biochar amendment, respectively.

### 3.4. Soil nutrients

In the soil without *Beta vulgaris*, the soil gained the highest AN content when 1% biochar was amended. Compared with the treatments without *Beta vulgaris* when biochar was applied, AN content was decreased. With *Beta vulgaris* planted, there was no significant difference in soil AN with 1% biochar amendment compared with no biochar treatment (Fig. 4a). As shown in Fig. 4b, whether planted *Beta vulgaris* or not, AP content increased with biochar amendment. In addition, AP increased to 276% in *Beta vulgaris* group amended with 5% biochar compared with no biochar treatment. There was no significant difference in soil LOC between 1% biochar amendment treatment and no biochar treatment whether planted *Beta vulgaris* or not, but 5% biochar amendment treatment gained LOC increased by 10% in the soil with *Beta vulgaris* planted (Fig. 4c). Fig. 4d also showed that the atom ratio of soil total C/N increased with dose of biochar increased, compared with the treatment planted *Beta vulgaris* without biochar, the soil C/N in the

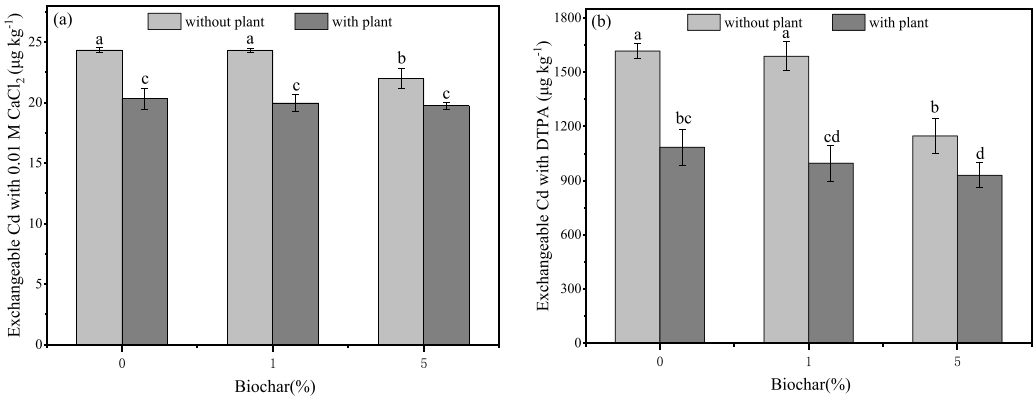


**Fig. 2.** Cd extracted by 0.01 M $CaCl_2$ (a) and DTPA (b) in soil with different treatment. Light grey: without *Beta vulgaris*; dark grey: with *Beta vulgaris*. 0, 1 and 5 meant the application at doses of 0, 1 and 5% (w/w) cornstalk biochar, respectively. (Data are mean ± SD, n = 3. Error bars denote the standard deviations. Different letters above the columns indicate statistically significant differences among treatments ($P < 0.05$)).

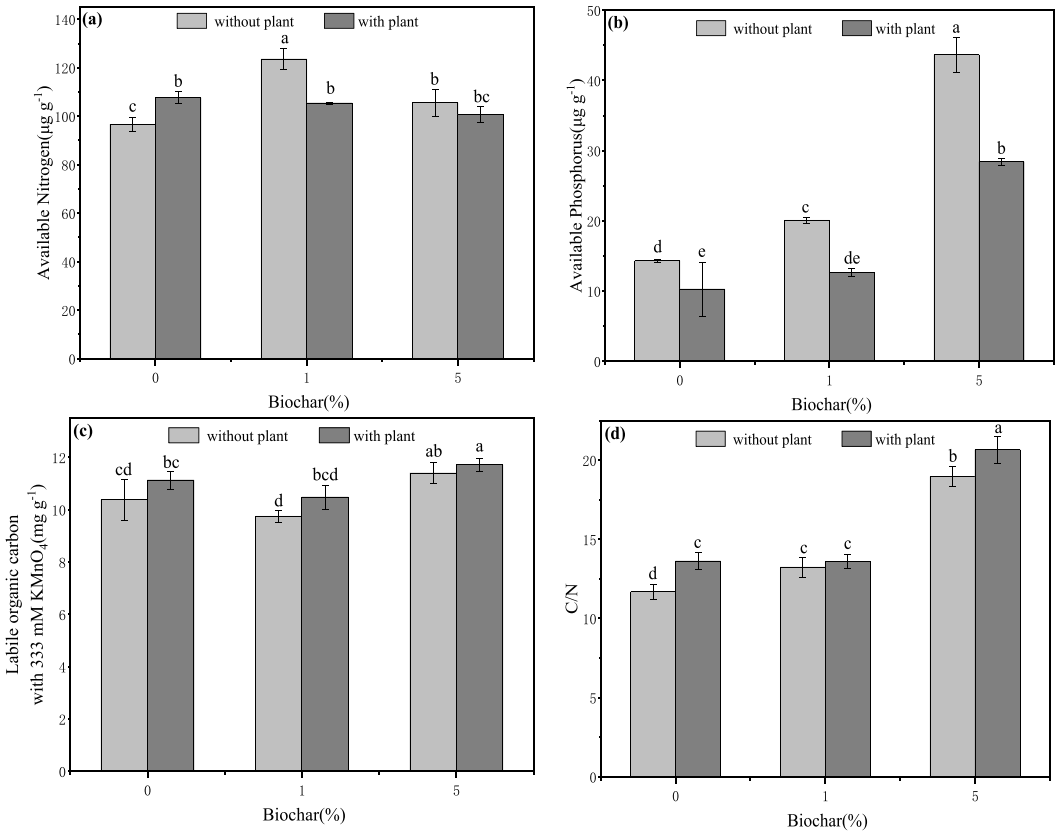


**Fig. 4.** Concentration of nutrients in soil with different treatment. Light grey: without *Beta vulgaris*; dark grey: with *Beta vulgaris*.(a): AN; (b): AP; (c): LOC; (d): atomic ratio of total C/N in soil. 0, 1 and 5 meant the application at doses of 0, 1 and 5% (w/w) cornstalk biochar, respectively. (Data are mean ± SD, n = 3. Error bars denote the standard deviations. Different letters above the columns indicate statistically significant differences among treatments ($P < 0.05$)).

5% biochar treatment group increased from 12 to 22.

### 3.5. *Beta vulgaris* growth

As exhibited in Fig. 5, the dry biomass of the root bred significantly as the dose of biochar increased. Root dry weight increased to 267% amended with 5% biochar. However, the addition of biochar reduced the above-ground biomass of 14–15% compared to the treatment without biochar.

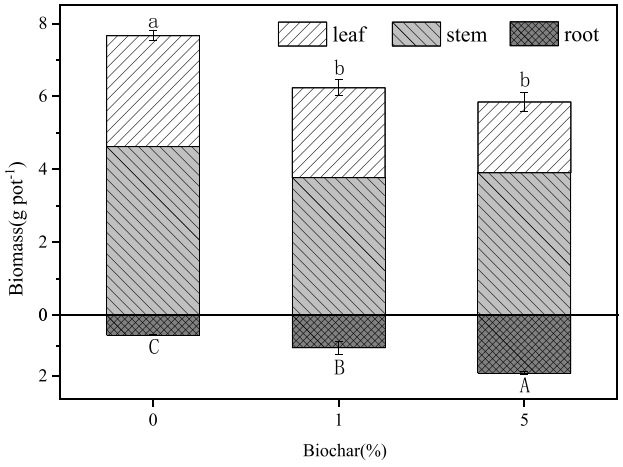


**Fig. 5.** The dry weight of *Beta vulgaris* with different treatment. 0, 1 and 5 meant the application at doses of 0, 1 and 5% (w/w) cornstalk biochar, respectively. (Data are mean ± SD, n = 3. Error bars denote the standard deviations. Different letters above the columns indicate statistically significant differences among treatments ($P < 0.05$)).

### 3.6. *Cd chemical forms in the root*

Different chemical forms of Cd and its percentage in *Beta vulgaris* root with different doses of biochar amendment are shown in Fig. 6a and Fig. 6b, respectively. As demonstrated in Fig. 6b, the NaCl-extracted Cd and HAc-extracted Cd were prominent. As shown in Fig. 6a, the total content of ethanol-extracted Cd and d–$H_2O$–extracted Cd decreased by 13% and 29% in the treatment of 1% and 5% biochar, respectively, compared with the treatment of no-biochar amendment. The total content of HCl-extractable Cd and residue Cd increased by 0.14 and 0.52 times in the treatment of 1% and 5% biochar, respectively. As manifested in Fig. 6b, the percentage of NaCl-extracted Cd in the treatment with biochar amendment was 1.38–1.42 times higher than that in biochar-free treatment, while the percentage of HAc-extracted Cd was 80–83% lower.

## 4. Discussion

### 4.1. *Available Cd and the fractions in soil*

The Cd extracted by $CaCl_2$ represented the mobility of Cd and was strongly correlated with soil pH (Chaignon et al., 2009). In the *Beta vulgaris*-planting groups, no significant difference was obtained about Cd concentration extracted by $CaCl_2$ among the treatments with different doses of biochar amendment (Fig. 2a). The result may be owing to that the soil pH did not change strongly (Table S1). The Cd concentration extracted by DTPA was significantly correlated with Cd concentration in crops. Accordingly, DTPA is often used to evaluate the immobilization

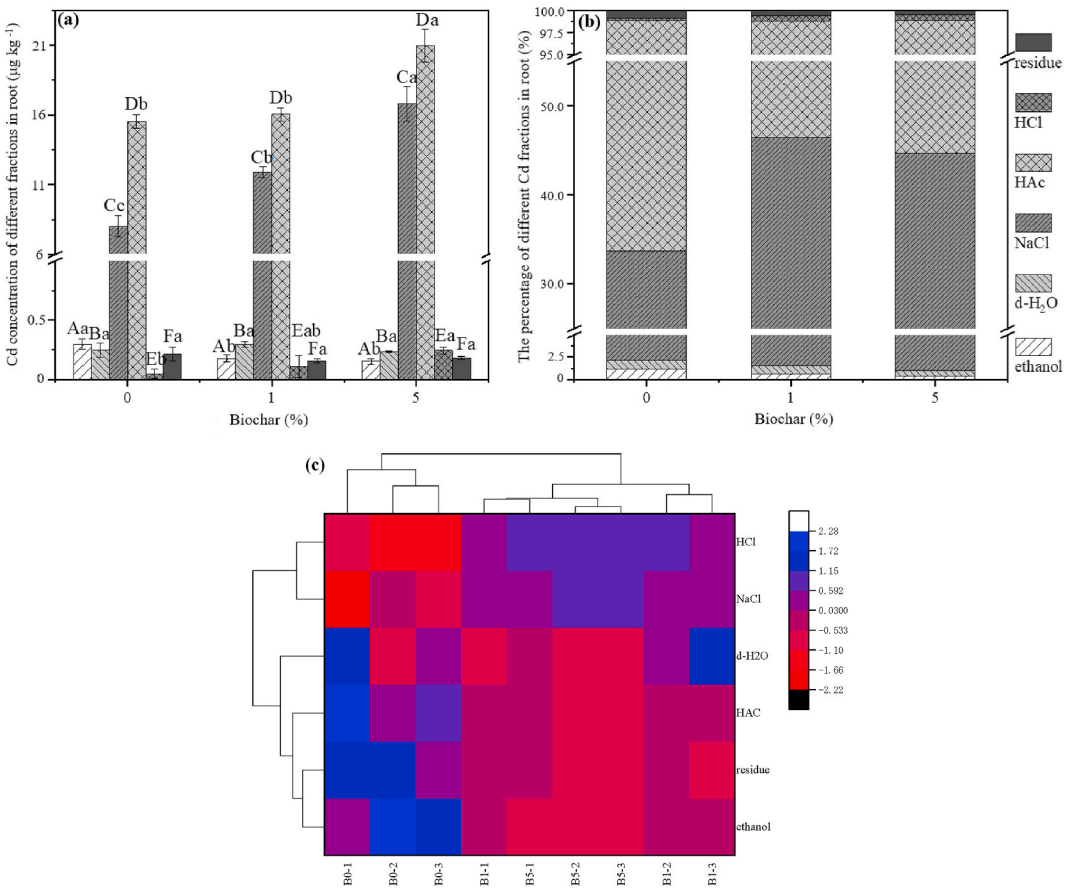


**Fig. 6.** Different forms of Cd and its percentage in *Beta vulgaris* root with different treatment. (a): the Cd concentration of different fractions in root; (b): the percentage of different Cd fractions in root. (c): heatmap showing forms of Cd in root with different treatment. The color weighting means normalized levels of each variable from low (red) to high (blue). 0 (B0), 1 (B1) and 5 (B5) meant the application at doses of 0, 1 and 5% (w/w) cornstalk biochar, respectively. 1, 2 and 3 meant three parallel groups of the same treatment. (Data are mean ± SD, n = 3. Error bars denote the standard deviation. Different small letters with the same capital letter indicate statistically significant differences among groups ($P < 0.05$)). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

exerted by biochar (Rehman et al., 2017; Zhu et al., 2016). As exhibited in SEM (Fig. S1), the cornstalk biochar had developed pores and large specific surface area. Biochar has a strong ability to adsorb heavy metals (Liu et al., 2017). The Cd concentration extracted by DTPA decreased significantly with amendment of 5% biochar because the Cd in soils was immobilized by biochar at the dose of 5%.

Both biochar and *Beta vulgaris* could affect the species of Cd in soils (Gong et al., 2018; Li et al., 2018c). Plants can secrete root exudates such as low molecular weight organic acids. By forming organic/amino acid-metal/mineral complexes or by acidification due to proton ($H^+$) release, root exudates increased soil nutrients and enhanced mobility of metals (Ma et al., 2016). Previous researches were also shown that hyperaccumulators root could induce dissolved organic matter (DOM) increase in rhizosphere under heavy metal stress and acid fractions were the main components in rhizosphere soil (Li et al., 2011, 2012). The roots were more developed in the 5% biochar treatment compared with the biochar-free treatments (Fig. 5). The concentration of Fe–Mn oxides-bound Cd decreased with the dose of biochar increased. This may due to the increase of root biomass, which cause more developed roots to release more acidic DOM and transform Fe–Mn oxide-bound Cd to other fractions which of higher bioavailability. Rechberger et al. (2019) suggested that Cd was predominantly bound as $CdCO_3$ on the biochar after 15 months (82.7%) and the formation of organic functional groups played a role for increasing Cd sorption on aged biochar. Relative to the treatment without biochar, the total content of organic matter-bound Cd and residual Cd increased in the 5% biochar treatment, in spite of root exudates mobilizing Cd. This may be attributed to the formation of sulfur or phosphoric acid precipitationes and the complexation or chelation of functional groups on the surface of biochar with Cd (Melo et al., 2015; Tan et al., 2017; Xu et al., 2018). To conclude, the Fe–Mn oxides-bound Cd was transformed into high stable fractions (organic matter bound Cd and residual Cd), and bioavailable Cd speciation which could be absorbed by *Beta vulgaris*.

### 4.2. Soil nutrients and plants Cd accumulation

Biochar is rich in nutrients (Mohamed et al., 2017; Zhao et al., 2016) such as C, N, P, etc., and the addition of biochar to soil also improved soil particle size distribution (Table S2), which affect soil physicochemical properties and plants growth (Huang et al., 2019). The AP, TN, TOC, LOC, C/N atom ratio, significantly increased in 5% biochar amended soil (Fig. 4b, Fig. S2; Fig. S3, Fig. 4c and 4d). The increase of soil nutrients could increase *Beta vulgaris* root biomass evidently (Fig. 5). At the same time, biochar amendment reduced Cd mobility (Fig. 2b), thereby reducing Cd toxicity to plant root and promoting root growth, which may lead to the increase of Cd absorption sites on root surface and promote the uptake of Cd to *Beta vulgaris*. These reasons may offset the effect of decrease Cd availability in biochar-amended soil and promote the accumulation of Cd in *Beta vulgaris*. This explanations were consistent with the findings of Rees et al. (2016) and Park et al. (2011). Moreover, the increased plant uptake of nutrients should have contributed to increased aboveground biomass. However, the aboveground

biomass of *Beta vulgaris* didn't increase after biochar addition (Fig. 5), it may be due to the toxic effects of heavy metals. Although the portion of bioavailable Cd content in soil decreased, the roots of plants absorbed more Cd and transported it to the aboveground leaves, which is similar to the results found in a previous study (Rees et al., 2016). Thus we conjectured that the addition of biochar will reduce the heavy metal content in common plants, but for accumulators, the lower available heavy metal content in soil will not affect them transfer heavy metals to aboveground parts.

Table S4 showed that the AP content was significantly correlated with dry weight and Cd content of roots (0.939, 0.726), and root biomass of *Beta vulgaris* was significantly correlated with TOC and TN (0.922 and 0.951). LOC was a crucial indicator of land productivity and responded to the changes of organic carbon in soil environment. LOC was extremely easy to be oxidized and mineralized by microorganisms, and was considered as a direct response to the supply of nutrients (Wang et al., 2005). The amendment of 5% biochar significantly increased LOC in the soil (Fig. 4c). This indicated that increased fertility of soil could provide more labile carbon source for microorganisms. Fig. 1d also showed that, as the biochar increased, the C/N in the soil increased, which was closer to the optimal C/N (25) for microbial activity (Spohn and Chodak, 2015).

Microorganisms can promote the absorption of contaminant by plant roots (Bell et al., 2014; Gong et al., 2019). Soil fluorescein diacetate activity (FDA) was recognized as an indicator for soil microbial activity (Jiang et al., 2016; Liu et al., 2011). The changes of soil microbial activity could be seen in Fig. S4, in the soil with *Beta vulgaris* planted, microbial activities increased 11.3% with 5% biochar amendment compared to the treatment without biochar. There was no significant influence on the activities of microorganisms in *Beta vulgaris* planted treatments whether added biochar or not. Microorganisms can stimulate plant growth directly by the solubilization of mineral nutrients (phosphate, nitrogen, etc.), and/or indirectly via secretion of specific enzymes and production of plant growth promoting phytohormones. Meanwhile, biochar abundant pore structures provided better habitats for microorganisms and nutrient supplementation could also reduce Cd stress on microorganisms to enhance microbial activity, thus promoting phytoremediation, despite the reduced availability of Cd in soils. This explanation is also mentioned in the literatures by Gong et al. (2019) and Lu et al. (2015). Also, microorganisms could change the bioavailability of metal via various mechanisms such as acidification, precipitation, chelation, etc. (Ma et al., 2016). Therefore, increased nutrients and microbial activity played a role of synergy effect to promote root growth, thereby increased Cd uptake of *Beta vulgaris*.

P is often adsorbed by Fe/Al oxides in soil, leading to low availability (Gu et al., 2016), nevertheless, the addition of biochar increased the AP content in soil (Fig. 4b), it could not only facilitate the *Beta vulgaris* root growth (Fig. 5), but also affect the chemical form of heavy metals in soils and plants (Figs. 3 and 6). For phosphate fertilizer application of soluble phosphate, the amendment often results in lower soil pH, which improves the availability of some metals. As Huang et al. (2013) have shown that Zn mobility in soils and uptake by hyperaccumulator *Sedum alfredii* were raised by phosphate fertilizer. Also, P application increased the concentration of exchangeable and carbonate-bound Zn in soils (Huang et al., 2019). In plants, higher P concentrations in tissues reduced Zn physiological availability and stress to plants, thus promoting plant growth (Cakmak and Marschner, 1987; Cao et al., 2018). In other relevant studies, Ngoc et al. (2018) and Zhao et al. (1998) found that P could alleviate the toxicity of Zn to hyperaccumulator *N. caerulescens* and *Thlaspi aerulescens* by forming precipitation of Zn and P in the root of the hyperaccumulators. *Beta vulgaris* was reported as a Cd hyperaccumulator in some studies (Chen et al., 2013; Song et al., 2012; Yushuang et al., 2007). Cd may precipitate with P in the root of *Beta vulgaris*, thereby reducing mobility of Cd in roots and its toxicity to *Beta vulgaris*. Additionally, as exhibited in Table S3, AP was significantly correlated with the Cd content in *Beta vulgaris* root (0.726), and P was the only element among nutrient correlated with root growth. Therefore, P may play a vital role in the uptake of Cd by *Beta vulgaris*.

### 4.3. Cd chemical forms in the root

As shown in Fig. 6, the NaCl-extracted and HAc-extracted Cd species were dominant, which indicated that the Cd in roots of *Beta vulgaris* was bound to pectates, protein and undissolved phosphate. It was generally believed that NaCl-extracted Cd bound to proteins and pectin, or adsorbed on protein surfaces (Pauly and Keegstra, 2016; Zhang et al., 2019). Moreover, pectin molecules made up 30% of the primary cell wall and were stressed as critical molecules to isolate divalent ions in the primary cell wall (Loix et al., 2017). Consequently, we speculated that Cd was mainly adsorbed on the *Beta vulgaris* root cell wall. Similar results were found by Fu et al. (2011) that Cd chemical forms in the *Phytolacca americana* L. were mainly pectin and protein associated, and the result of subcellular distribution indicated that Cd was predominantly in the cell wall. Another research revealed that the accumulation of Cd in the root cell wall could reach even higher level and almost all absorbed Cd was accumulated in *Dittrichia viscosa* L. root cell walls (Fernandez et al., 2014).

The chemical speciation of heavy metals in plants corresponds with their biological function (Zhang et al., 2019). The toxicity and distribution of Cd in *Beta vulgaris* roots were indicated with the set of extraction solutions applied. The total content of ethanol-extracted Cd and d–$H_2O$–extracted Cd decreased, and the total content of HAc-extracted Cd and residue Cd increased in the treatments of 1% and 5% biochar, compared with the treatment of no-biochar (Fig. 6a). Therefore, the toxicity of Cd to *Beta vulgaris* was reduced by biochar. A high proportion of NaCl-extractable Cd was interpreted as the adaptation of the plant to Cd stress induced high Cd accumulation capacity (Fu et al., 2011). With biochar amendment, the proportion of NaCl-extracted Cd significantly increased and the proportion of HAc-extracted Cd was reduced compared with the biochar-free treatment, which may be one of the mechanisms of *Beta vulgaris* resistance to Cd after biochar amendment. Consequently, the potentiality of Cd accumulation in *Beta vulgaris* was enhanced.

Fig. 6c manifested the hierarchical clustering analysis of Cd chemical forms relative abundances in *Beta vulgaris* roots using the average linkage distance among clusters. The color weighting means normalized levels of each variable from low (red) to high (blue). It showed the accumulation characteristics of *Beta vulgaris* amended with biochar were significantly different from the *Beta vulgaris* without biochar. It indicated that biochar changed the accumulation pattern of *Beta vulgaris*. The Cd concentration of *Beta vulgaris* amended with 1% biochar (26.52 mg $kg^{-1}$) was close to the concentration of *Beta vulgaris* in the biochar-free treatment (25.38 mg $kg^{-1}$), but the ratio of NaCl-extracted Cd to HAc-extracted Cd in treatment of 1% biochar group accounted for 0.86, while the treatment without biochar only accounted for 0.49. The proportion of NaCl-extractable Cd was significantly improved. Krzeslowska et al. (2016) found that thickening of pectinous cell wall was a common defense strategy for plants. When plants faced stress of heavy metals, biochar may enhanced the defense strategy by inducing pectinous cell wall thickening. Moreover, we guessed that the larger the concentration ratio of NaCl-extracted to HAc-extracted Cd in the plant may be, the more appropriate it is to combine with biochar in remediating Cd-contaminated soil. Whether this assumption was true or not, we will carry out related experiments in the follow-up.

In this study, we considered that the type of remediation plant is a crucial factor affecting the joint effect of plants and biochar and the effects of biochar on different plant types may be different. The plants need to be capable of accumulating Cd mainly into cell wall. A future comparison of the Cd chemical forms in the leaf and root is also were needed to confirm the outstanding role of the proportion of NaCl-extracted Cd.

## 5. Conclusions

In summary, Cd accumulation increased significantly in *Beta vulgaris* with 5% biochar amendment. Biochar as soil amendment increased the soil AP, LOC, TN, TOC, C/N atom ratio and promoted microbial activity and root biomass production, then phytoextraction was enhanced. Cd availability extracted by DTPA reduced after 5% biochar was applied to soil. Meanwhile, the total content of organic matter-bound Cd and residual Cd increased and the content of Fe–Mn oxides-bound Cd decreased. In addition, results suggested that Cd mainly integrated with pectates, protein and undissolved phosphate in *Beta vulgaris* root after biochar amendment. Protein and pectin constituents were the major binding sites of Cd in the cell walls of roots, thereby we speculated that Cd was mainly in the root cell wall. A higher proportion of Cd adsorbed in the cell wall of *Beta vulgaris*, which may be one of the mechanisms of *Beta vulgaris* resistance to Cd after biochar amendment. In the light of our research, Cd in soils could be removed by *Beta vulgaris* and phytoremediation efficiency could be improved by biochar amendment. This study provides a favorable data support for the application of biochar combined with accumulators to remediate Cd contaminated soil and proves this joint strategy is feasible and promising. However, more specific and in-depth mechanisms of joint effects still need to be explored in the near future.

## CRediT author statement

**Panxue Gu:** Methodology, Investigation, Formal analysis, Writing - original draft. **Yanming Zhang:** Methodology, Investigation, Validation. **Huanhuan Xie:** Validation, Data curation. **Jing Wei:** Conceptualization, Resources, Supervision, Project administration. **Xinying Zhang:** Conceptualization, Resources, Writing - review & editing, Supervision, Funding acquisition. **Xun Huang:** Visualization, Writing - review & editing. **Jiayi Wang:** Writing - review & editing. **Xinyi Lou:** Writing - review & editing.

## Declaration of competing interest

We declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence our work, there is no professional or other personal interest of any nature or kind in any product, service or company that could be construed as influencing the position presented in, or the review of the manuscript submitted.

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## Appendix A. Supplementary data

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