Effect of biochar on tungsten bioavailability and uptake grown in a soil with artificially tungsten contaminated

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GRAPHICAL ABSTRACT

To explore the migration and transformation of W-biochar-plants

Different BC levels (0%, 1%, 2% and 5% w/w)

Effects of BC on soil  Effect of BC on plant parameters  Effect of BC on lettuce enrichment W

Abstract:

Tungsten (W) has become an emerging pollutant due to its potential toxicity to the human body, and biochar (BC) is widely used for the remediation of heavy metals in soil. However, W—
biochar's interaction with plants remains unknown. This trial aimed to investigate the effect of rice husk BC on the absorption of lettuce W. Add different levels of BC (0%, 1%, 2%, and 5% w/w) to the soil and incubate for 60 days. The results show that the mechanism by which BC application fixes W in soil and converts the acid extractable and reducible parts into residual parts has not been studied. At the same time, BC treatment improved the stem height, root length, biomass and chlorophyll content of lettuce compared with the control. Compared with the control, the accumulation of W treated with 5% BC was reduced in both the aboveground and roots, and the transport factor (root-to-aboveground) was the lowest. The results show that the main benefit of BC is reduced W to proximal water mobilization. In addition, the administration of BC can effectively fix and reduce the absorption of W by lettuce.

Keywords: Tungsten; Bioavailability; Biochar; Stabilization

1. Introduction

Tungsten (W) is a precious rare metal that has been widely used in a wide range of applications from everyday household necessities to high-tech industries (preliminary risk concentrations of W in soil and drinking water, land contamination census). The cancer case report in western Nevada brought W to the scientific attention of the academic community and detected a high incidence of acute leukemia (Schell and Pardus 2009). To assess the environmental causes of leukemia clusters, several studies have been conducted. Finally, W is considered an environmental factor in leukemia due to the higher concentration of W in urine and drinking water samples. Since then, W has been identified as an emerging contaminant (Datta et al. 2009).
Soil can be contaminated with high concentrations of W due to human activities such as industrial and agricultural activities, and mining activities are also one of the main sources of W in soil (Kennedy et al. 2012). Due to its complex chemistry, W behaves in soil quite differently than other metals. Due to the diversity of oxidation states (-2 to +6) and coordination numbers (5 to 9) of W, its chemistry is one of the most variable and complex of transition elements (Koutsospyros et al. 2006). W is an essential element of some microbial enzymes, but is not necessary for plants, animals and humans. In addition, W reduces germination rate, inhibits rhizome growth, destroys cells, and leads to cell death. Aging soil bioassays showed that cabbage growth was impaired when soil W levels were 436 mg/kg (Adamakis, Panteris, & Eleftheriou 2012). W is absorbed by the root system and transported through the xylem, and the accumulation of W in the root system is much higher than in the stem. The accumulation of W of several plants in abandoned mines was studied, and the W content of digitalis purpurea was 90.8 mg/kg (Pratas et al. 2005).

Stabilization technology is a widely used method for remediating metal-contaminated soil. BC has been widely used for heavy metal stabilization (Lamori et al., 2017). While there have been some studies that have looked at the uptake of W by plants, the effects of soil remediation agents on it are unclear. Unlike many other metals, which have been well documented as contaminants that can be cured by BC, existing knowledge about W stabilizers is relatively limited (Zheng et al. 2020). Therefore, it can be a productive topic for discussion. In addition, lettuce is a common vegetable that grows rapidly and reacts to metal pollution in the soil (Kim et al. 2015). On this basis, lettuce was used as the research object, and the effect of BC on soil W
and the absorption of W by lettuce were studied.

2. Materials and methods

2.1. Materials collection and characterization

All experimental chemicals were analytical grade and used as received. Sodium tungstate (Na₂WO₄) was selected as the W source. The field soil was collected from garden in Jiangxi University of Science and Technology in China. Soil properties are followed below: 19.17% moisture (by drying to constant weight at 105°C), 2.47% soil organic materials (by loss on ignition at 550 °C for 4 h), pH was 5.96 (in a soil:water ratio 1:2.5). Before the experiment, the air-dried soil was broken and pass through a 2 mm sieve for saving.

BC was produced by pyrolysis of rice husk at 400 °C in the absence of O₂ (4 hours), and broken to pass through a #60 sieve after oven drying. The pH of BC (1:20, w/v, weight to distilled water ratio) were measured using pH meters (Abbas et al. 2017). Like most studies, BC was alkaline (pH 7.81), and with many major nutrients (Table1). Thus, BC may have high reactivity to cations because of its high BET (Brunauer-Emmett-Teller) surface area (89.06 m²/g) and negative Zeta potential (-28.82 mV) (Meier et al. 2015). Conversely, it may have lower reactivity to W which was existed in the soil in the form of oxyanions.

Fig. 1 depicts the functional groups of BC, which were identified by FTIR spectroscopy. At the range of 3,500 to 3,100 cm⁻¹, the absorption is assigned to the stretching vibration of N-H bonds from amines and amides (Liu et al. 2018). The peak at 1600 cm⁻¹ was related to C=O stretching attributed to the carbonyl group (Bamdad and Hawboldt 2016). The peak near at 1100 cm⁻¹ indicate the presence of C-N from tertiary amine. The band at 800 is due to the presence of —
(CH$_2$)$_n$— (n $>$ 4), and indicate that long carbon chain was existed.

2.2. Immobilization assay

A group of soil was prepared individually to evaluate the effect of application dose of BC for extractable rate and observe the metal forms transformation of W during the treatment period. The field soils were spiked with Na$_2$WO$_4$ solution. So the concentration of W was 60±1.24 mg/kg, fully mixed up and incubated for 14 days before air drying. Each plastic pot (height 20 cm, top and bottom diameter of 18 cm and 15 cm respectively) was filled with 3 kg of soil on dry weight basis. BC was added at application rate of 0% (CK), 1.5%, 3.0%, and 5.0% w/w in the soil and fully mixed and then incubated for 60 days at 25 ± 2 °C. The soils were kept 60% of field capacity, which corresponds to 15-18% of soil moisture content that was monitored using a digital thermo-hygrometer equipped with a sensing probe. All experiences were triplicated.

2.3. Soil analysis of immobilization assay

After 60 days incubation of immobilization assay, 200 g soil samples were collected for property analysis. Soil pH was measured using a pH meter at a soil/water ratio 1:2.5. The ammonium acetate extraction procedure was used for cation exchange capacity (CEC) determination (Lusiba, Odhiambo and Ogola 2016). Soil organic carbon (SOC) determined using the Walkley and Black method (Nelson and Sommers 1996). The Stuanes method was used for exchangeable acidity determination (Stuanes, Ogner and Opem 1984). CaCl$_2$ extract procedure was carried out to evaluated the the stabilization rate of BC on W, which method is closely related with contaminant bioavailability to soil organisms (Cao et al. 2011). Meanwhile, BCR sequence extract procedure was adopted to determine metal form present in soil sample
after BC application (Davidson et al. 1998). The concentration of W in soil leaching agent was determined by ICP-MS (Agilent8800, SureCycler). The following is a list of the sequence extraction procedures performed on the soil W:

F1 - acid extractable fraction. 1.0 g soil (through a #100 sieve) was extracted with 40 ml of 0.11 mol/L acetic acid with continuous shaking for 16 h at 25 ℃. Then, centrifuging at 3000 r/min for 2h at room temperature, filtering the supernatant fluid, and saving for the next step of the experiment.

F2 - reducible fraction. The residual of F1 was washed with deionized water and extracted with 40 ml of 0.5 mol/L hydroxylamine hydrochloride, with continuous shaking for 16 h at 25 ℃. Then, centrifuging at 3000 r/min for 2h at room temperature, filtering the supernatant fluid, and saving for the next step of the experiment.

F3 - oxidizable fraction. The residual of F2 was washed with deionized water and extracted with 10ml hydrogen peroxide with pH 2-3, continuous digesting for 1 h at room temperature and 1 h at 85 ℃, respectively. Add 50 ml 1 mol/L ammonium acetate for extraction with continuous shaking for 16 h at 25 ℃. Then, centrifuging at 3000 r/min for 2h at room temperature, filtering the supernatant fluid, and saving for the next step of the experiment.

F4 - residual fraction. The residual of F3 was washed with deionized water and digested as procedure of total metal analysis.

2.4. Pot experiment

A further treatment, after 60 days incubation, a pot experiment was conducted to evaluate the effect of BC application on lettuce. Before pot experiment, soil was air-dried, ground and passed
through a 1 mm sieve. Initially, 10 lettuce seeds were sown in each pot (height 9.6 cm, top and bottom diameter of 17 cm and 12.3 cm respectively) containing 1.5 kg of soil. The seeds were germinated for 7 days, then the plants were thinned to 1 per pot. And then, the lettuce was cultivated for 60 days with a 16 h photoperiod at 25 ± 2 °C, and kept 60% of field capacity. Both germination and cultivation were carried out in growth chamber. Therefore, in total there were 12 pots, giving 4 triplicated treatments, 0% BC, 1% BC, 2% BC and 5.0% BC.

2.5. Plant analysis of pot experiment

Further, after 60 days growth, the plants were harvested, and initially washed thoroughly with tap water followed by deionized water and recorded plant biomass. Then, the lettuce were separated into roots and shoots lengths were measured using a measuring scale, shoot and root samples were freeze-dried and ground to powder. To analyze W uptake by lettuce, 0.2 g of ground shoot and root samples were digested using 15 mL of concentrated HNO₃ and closed-vessel microwave digestion procedure was performed (Park and Han 2019). The values of W in digestate were determined using an inductively coupled plasma mass spectrometry (ICP-MS, Agilent-8800).

3. Results

3.1. Immobilization assay

3.1.1. Effects of BC on the properties of soil

The application of BC significantly changed the physicochemical properties of the soil such as pH, exchange acidity, organic carbon and CEC. Soil pH significantly influences the mobility of W (Bednar et al. 2009). The application of BC causes a change in soil pH, which is related to
Numerous studies have shown that BC can be used as an excellent acidic soil additive to increase soil pH to fix heavy metals. Organic carbon content is an important indicator of soil fertility and can compete with oxygen anions for retention points. Organic carbons, including humic acids, organic clays, and oxides coated with organic matter, have a high affinity for metals (Ghosh and Singh 2005). Therefore, the increase of SOC after BC application may be one of the reasons for the change in W mobility. CEC is an important factor affecting the retention and migration of metals in soil, which is related to the adsorption capacity of soil to metals.

Soil exchange acidity decreases with the increase of BC application. The acidity of soil colloidal adsorption exchangeability H\(^+\) and Al\(^{3+}\) is called exchangeable acidity, which represents the acidification trend of the soil. Therefore, exchange acidity is an important parameter for the remediation of acidic soils. The effects of different BC application rates on soil parameters were observed (Table 2). Soil pH increases with the increase of BC application, which is mainly due to the alkalinity of BC, which is affected by different raw materials and production processes. Compared with the control group (CK), soil pH increased by 0.03, 0.08 and 0.18 units, respectively, 2% BC and 5% BC (p <0.05), and 1% BC had no significant effect on soil pH (p >0.05). After administration of 1%, 2%, 5%, and 10% BC, the exchange acidity decreased by 0.14, 0.30, and 0.81 cmol/kg, respectively, compared to the control group. In addition, BC is also an organic fertilizer with high organic matter and CEC content. The administration of 1% ~ 5% BC can increase SOC and CEC by 15.42% ~ 82.43% and 9.11% ~ 42.02%, respectively. After BC application, soil pH increased 0.03—0.18 units, and exchangeable acidity
decreased 0.14—0.81 cmol/kg, which was attributed to the high alkalinity of the BC displaying a liming effect (Rizwan et al. 2019). Carbonate contents and functional groups (such as — COO—, —O—) in BC surface were increased during the BC production process, which attributes to the pyrolysis of organic matter (Yuan, Xu and Zhang). Rice-husk-derived BC is rich in base ions, phosphates, carbonates, and metal oxides, which reduce the exchangeable acidity in the soil by adsorption to protons (Li M K and 2018). It can be concluded that the BC from rice-husk could be used as soil amendments to adjust soil acidity for its alkalinity. BC application significantly increased soil CEC, and the highest values of CEC were recorded at 5% of BC application rate. The increased CEC could be regarded as the high surface area, porous structure and organic matter contents of BC (Sun and Lu 2014).

3.1.2. Effect of application rate of BC on W stabilization (CaCl2 extraction results)

The stabilization rate of W was determined by the results of the TCLP tests. The stabilization rate of W was calculated according to the following formula:

\[ \text{Stabilization rate} = \left( \frac{C_0 - C_1}{C_0} \right) \times 100\% \]

Where \( C_0 \) is the extraction concentration of W in BC-untreated soil (CK), \( C_1 \) is the extraction concentration of W in after different BC application. With BC-untreated, the extraction concentration of W is 448.11±1.10 μg/L. The effect of the application rate of BC on the W stabilization rate is shown in Fig. 2. When the BC application rate increases from 0% to 5%, the stabilization rate of W was increased obviously. After 5% BC application, the extraction of W concentration in the soil was reduced to 210.56±1.10, and the stabilization rate of W reached 53.13% after 5% BC application. It can be figured out that 5% BC application has a high
The high metal immobilization rate was observed after BC application, which was primarily due to BC application induced increases in soil pH. Many previous studies are shown that pH could be the most important soil factor influencing metal mobility (Krol, Mizerna and Bozym 2019, Sakanakura et al. 2011, Cappuyns et al. 2013). According to our previous study, the leaching ability of W was generally increased with pH at a range of 3-12, while it's slightly decreased with pH increased from 6 to 7 (unpublished). SOC content is known to effect metals mobility. Its attribute to BC has influences on the negative charge of soil surface and chemical reactions between metals species, such as As which existed in the form of oxyanions. Lin et al. reported that BC application could improved As adsorption ability of red soil (Lin et al. 2018). It could be concluded that BC prompted the adsorption of W onto soil particles. Besides, increased SOC content increased the formation of metal-organo complexes, because W is capable of forming a large number of complexes with a variety of organic ligands. Recent research (Plattes et al. 2007) showed that primary amines in lysine can chelate with tungstate, which figured out that a strong interaction between primary amines and tungstate ions. Tong et al. reported that adsorption on metals via formation of surface complexation is mainly mechanism for biochars made from crop residues (Tong et al. 2011).

FTIR analysis indicates that the surface of BC contains primary amines, which may promote the diffusion of tungstate to BC pores, and accelerate the stabilization. CEC may play an important role in metal-oxyanions stabilization, especially Ca$^{2+}$. According to previous study, BC, load with calcium, can decrease the mobility of As. In this study, BC was not load, but high calcium
was observed, which dominated by its feedstock. Generally, immobilizing effect was controled by the complexation, surface adsorption and precipitation. Simultaneously, the these processes are enhanced by pore structure of biochar, its attribute to the slow water percolation (Downie, Crosky and Munroe 2012).

The possible immobilization mechanism is as follows: BC improves the adsorption capacity of W in soil particles by affecting the negative charge on the soil surface; hformation of surface complexation. Surface functional groups can stabilize W by surface complexation. W exists in the form of oxygen anions. W can be precipitated with mineral elements such as calcium in biochar. However, its stability mechanism needs to be further studied.

3.1.3. Transformation of W forms after BC-treated (BCR results)

The percentage of each fraction of W in soil samples with different BC application rate is performed (Fig. 3). In Fig. 3, “CK” shows the percentage with BC-untreated, while, “1% BC”, “2% BC”, “5% BC” show the percentage with different BC application rate. When natural soil samples were spiked with a high content of W (sodium tungstate), the largest amount of W presented in the oxidizable fraction (42.67%), which is supposed to be quite environmentally friendly, while, its amount was also high in the acid extractable fraction (35.80%). The percentage of reducible fraction and residual fraction were 14.93% and 6.60%, respectively. After 1% to 5% BC application, the residual fraction increased significantly, while the acid extracted fraction decreased. Compared to the control group, 1%, 2%, and 5% BC application rate increased the residual fraction to 17.40%, 31.38%, and 42.40% respectively, and acid extracted fraction decreased to 18.44%, 12.10% and 9.86% respectively. Furthermore, after BC
application, a slight decrease in the reducible state was observed. It can be figured out that most acid extracted fraction was converted into residual fraction, which was deemed to be quite environmental friendly like oxidizable fraction.

The mobility of W is strongly controlled by Fe (III) oxide/oxyhydroxide, and it was positively correlated with the content of Fe (III) oxide/oxyhydroxide (Johannesson et al. 2013). Macro, micro, and nanoporous structures in biochar may provide reducing conditions for Fe and Mn (Joseph et al. 2010, Lin et al. 2012). When the redox potential is negative, Fe$^{3+}$, Mn$^{4+}$ is reduced to Fe$^{2+}$, Mn$^{2+}$, the adsorption of W on Fe (III) oxide/oxyhydroxide was decreased. This process could be a reasonable explanation for the slight reduction in the reducible fraction.

Besides, high content of dissolved organic matter (DOM) entered the soil. After that, W could complex with DOM and convert to oxidizable fraction. Beesley (Beesley et al. 2013) suggested that biochar was a source of Fe (II), which could precipitate with tungstate ion. Tungstate polymerization reactions are generally favored at lower pH (Bednar et al. 2008). Control group soil sample has a lower pH (6.13) and a higher acid extractable fraction (35.80%), we can regard that polytungstate has a higher mobilization in this soil sample. After BC application, the increased pH leads to depolymerization of polytungstate, and tungstate was produced. For negative redox potential conditions, Fe$^{2+}$ and Mn$^{2+}$ were produced and precipitate with tungstate. The reason for increases of the residual fraction was considered as follows: the produced Fe$^{2+}$ and Mn$^{2+}$ were precipitate with tungstate, and increased SOC increased the absorption of W on soil particles.

3.2. Pot experiment
3.2.1. Effect of BC on plant parameters

The growth and biomass of lettuce were significantly increased by BC application (Fig. 4). 5% BC application increased lettuce fresh biomass, shoot height, and root length by 32.29%, 45.33%, and 61.54%, respectively, compared to the control group. The average shoot height and root length were increased to 11.6 ± 0.57 cm and 10.9 ± 1.15 cm per plant compared to 7.8 ± 0.42 cm and 7.5 ± 0.57 cm per plant in the control treatment. Also, BC application significantly (p≤0.05) increased lettuce biomass, which was 8%, 15%, and 32% higher after the application of 1%, 2%, and 5% BC treatments compared to the control. Compared to the control group, the average chlorophyll contents of lettuce were increased to 0.75±0.14, 1.05±0.01, and 1.45±0.21 mg/kg with 1%, 2%, and 5% BC application.

The present research indicates that BC application has a positive effect on lettuce growth and photosynthesis compared to the control group (Fig. 5). Some research has shown that BC could increase plant growth and photosynthesis under metal-stressed species (Rizwan et al. 2016, Younis et al. 2016). Unfortunately, the interaction of W-BC-plant remains unknown. In this study, the shoot height, root length, biomass, chlorophyll of lettuce was increased with increased BC dose; this can be regarded as the increase in the mineral nutrient of BC (Table 1), and decreased W concentration in the plant (Fig. 6). W has toxic attributes to suppression of seedling, reduction of root and shoot biomass, aberration of cell cycle, disruption of the cytoskeleton and programmed cell death(Adamakis et al. 2012). However, the mechanism by which W exerted this effect has not been studied. The elongation of root inhibited by W may attributed to ultrastructural defects caused by W (Adamakis, Panteris and Eleftheriou 2011). The
W in soil was immobilized by BC (Fig. 2 and Fig. 3), which could reduce W availability. Besides, the nutrient elements contained in BC have positive effects on plant growth (Rafique et al. 2019, Kumar et al. 2019), including potassium, nitrogen and phosphorus. In addition, BC, produced in low pyrolysis temperature, is rich in DOM. In Bian et al study, they regarded that BC was exerting a more significant improvement on plant biomass, which attribute to large DOM (Bian et al. 2019).

### 3.2.2. Effect of BC on lettuce enrichment W

BC application increased average W content in lettuce shoot and root (Fig. 6). While, the effects of 1% and 2% BC were not obvious, 5% BC application was significantly increased W content. W concentrations in lettuce shoot and root ranged from 3.72 mg/kg to 5.34 mg/kg and from 24.94 mg/kg to 30.45 mg/kg, respectively. Average translocation factor (W content in shoot / W content in root) of W from root to shoot was 0.1791 for lettuce with 0% BC application and 0.1509 for lettuce with 5% BC application, showing significant difference between the two. Simultaneously, 1% and 2% BC application decreased W uptake for shoot and root, but there was no significant change in W translocation factor (0.1791 and 0.1795 respectively). The root of lettuce is not edible portion. Thus, decrease of W translocation from root to shoot with BC application indicates that BC can be used to enhance food security and enhance phytoremediation of W contaminated soils. The enrichment factor (W concentration in plant / W concentration in soil) of control group was 0.09 for shoot and 0.49 for root. After 5% BC application, the enrichment factor of shoot and root was decreased by 0.08 and 0.48, respectively.
According to the enrichment factor of control group (0.09 for shoot and 0.49 for root). This suggests that most of the W in lettuce accumulates in the roots, which is consistent with previous research (Park and Han 2019). Elevated concentrations of heavy metals in the root system indicate that plants may reduce transport to buds through localized metals in tissues (Rizwan etal 2012).

4. Conclusion

Rice husk biochar can fix W in W contaminated soil, reduce effective W (cacl2 extractable), and increase soil pH, organic matter and CEC. The highest dose of BC (5%) is most effective. The application of BC promoted the growth and photosynthesis of lettuce, and increased the height of bamboo shoots, root length, biomass and chlorophyll content. The results suggested that the main profit of BC to reduce mobilizing of W to proximal water. In addition, the application of BC was effective in W immobilization and reducing uptake to lettuce.

Ethical Statement

All authors confirm that the research meets the ethical guidelines, including adherence to the legal requirements of the study country.

Competing interests

There are no Competing interests.

Acknowledgements

This work was supported by the [National Key R&D Program of China] under Grant [authorization number 2019YFC1805100]; [the National Natural Science Foundation of China] under Grant [No. 21767012]; and [the Ganzhou Science and Technology Innovation Talent]
Program] under Grant [2018] No. 50.


Table 1  Characteristics of the rice husk derived biochar (BC)

<table>
<thead>
<tr>
<th>Property</th>
<th>BC</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>7.81</td>
</tr>
<tr>
<td>Organic matter (g/kg)</td>
<td>485.77</td>
</tr>
<tr>
<td>Total C (%)</td>
<td>45.15</td>
</tr>
<tr>
<td>Total N (%)</td>
<td>1.01</td>
</tr>
<tr>
<td>P (g/kg)</td>
<td>0.96</td>
</tr>
<tr>
<td>K (g/kg)</td>
<td>27.10</td>
</tr>
<tr>
<td>Ca (g/kg)</td>
<td>1.4</td>
</tr>
<tr>
<td>CEC (cmol/kg)</td>
<td>35.32</td>
</tr>
<tr>
<td>BET (m²/kg)</td>
<td>89.06</td>
</tr>
<tr>
<td>Fe (mg/kg)</td>
<td>0.98</td>
</tr>
<tr>
<td>Zeta potential (mV)</td>
<td>-28.82</td>
</tr>
</tbody>
</table>

Note: BET, Brunauer-Emmett-Teller specific surface area
### Table 2  The effects of different BC application rate to soil parameter

<table>
<thead>
<tr>
<th>Treatment</th>
<th>pH</th>
<th>exchangeable acidity (cmol /kg)</th>
<th>SOC (g/kg)</th>
<th>CEC (cmol /kg)</th>
<th>Bulk density (g/cm³)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0% BC</td>
<td>6.13±0.02&lt;sup&gt;c&lt;/sup&gt;</td>
<td>4.25±0.02&lt;sup&gt;a&lt;/sup&gt;</td>
<td>26.80±0.08&lt;sup&gt;d&lt;/sup&gt;</td>
<td>10.78±0.32&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.25±0.03&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>1% BC</td>
<td>6.16±0.01&lt;sup&gt;c&lt;/sup&gt;</td>
<td>4.11±0.01&lt;sup&gt;b&lt;/sup&gt;</td>
<td>31.04±1.12&lt;sup&gt;c&lt;/sup&gt;</td>
<td>11.22±0.17&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>1.24±0.04&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>2% BC</td>
<td>6.21±0.01&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.95±0.06&lt;sup&gt;c&lt;/sup&gt;</td>
<td>34.51±0.50&lt;sup&gt;b&lt;/sup&gt;</td>
<td>11.58±0.23&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.24±0.06&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>5% BC</td>
<td>6.31±0.03&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.44±0.04&lt;sup&gt;d&lt;/sup&gt;</td>
<td>42.36±3.13&lt;sup&gt;a&lt;/sup&gt;</td>
<td>12.45±0.18&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.22±0.04&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>
Fig. 1  FTIR spectra of the biochar
Fig. 2  Effects of BC application rate on tungsten stabilization rate
Fig. 3  The percentage of each fraction of W with different BC application rate
Fig. 4 Effect of increased biochar application on lettuce shoot height, root length and fresh biomass. Bars represent standard deviation of three replicates. Different LSD letters indicate significant differences among biochar application at $p \leq 0.05$. 
Fig. 5 Effect of biochar application on tungsten distribution in lettuce shoot and root. Bars represent standard deviation of three replicates. Different LSD letters indicate significant differences among BC application at p≤0.05.
Fig. 6  Tungsten leaching content of different biochar application dose. Bars represent standard deviation of three replicates. Different LSD letters indicate significant differences among BC application at p≤0.05.