Animal carcass- and wood-derived biochars improved nutrient bioavailability, enzyme activity, and plant growth in metal-phthalic acid ester co-contaminated soils: A trial for reclamation and improvement of degraded soils

Hanbo Chen [a], Xing Yang [a,b], Hailong Wang [a,c*], Binoy Sarkar [d], Sabry M. Shaheen [b,e,f], Gerty Gielen [g], Nanthi Bolan [h], Jia Guo [i], Lei Che [j], Huili Sun [k], Jörg Rinklebe [b,l]

[a] *Biochar Engineering Technology Research Center of Guangdong Province, School of Environmental and Chemical Engineering, Foshan University, Foshan, Guangdong 528000, China*

[b] *University of Wuppertal, School of Architecture and Civil Engineering, Institute of Foundation Engineering, Water- and Waste Management, Laboratory of Soil- and Groundwater-Management, Pauluskirchstraße 7, 42285 Wuppertal, Germany*

[c] *Key Laboratory of Soil Contamination Bioremediation of Zhejiang Province, Zhejiang A&F University, Hangzhou, Zhejiang 311300, China*

[d] *Lancaster Environment Centre, Lancaster University, Lancaster, LA1 4YQ, UK*

[e] *King Abdulaziz University, Faculty of Meteorology, Environment, and Arid Land Agriculture, Department of Arid Land Agriculture, 21589 Jeddah, Saudi Arabia*

[f] *University of Kafrelsheikh, Faculty of Agriculture, Department of Soil and Water Sciences, 33 516 Kafr El-Sheikh, Egypt*

[g] *Scion, Private Bag 3020, Rotorua 3046, New Zealand*

[h] *Global Centre for Environmental Remediation, University of Newcastle, Newcastle, NSW, 2308, Australia*

[i] *Chengbang Eco-Environment Co. Ltd, Hangzhou, Zhejiang 310008, China*

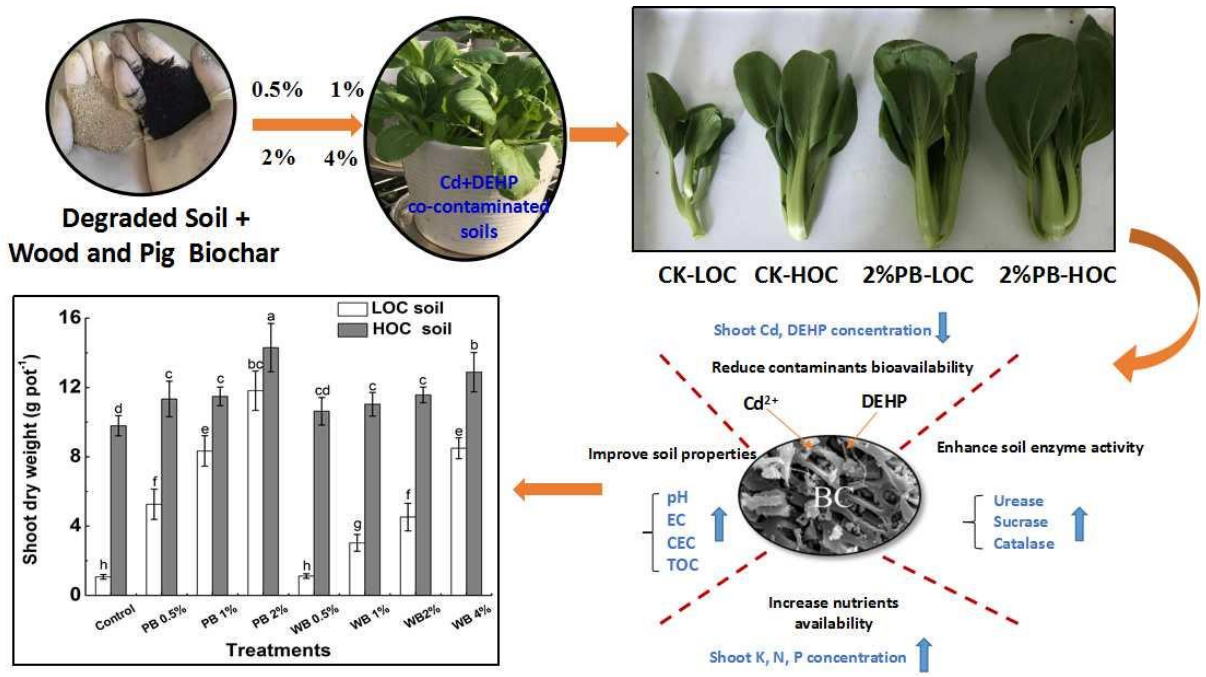
[j] *School of Engineering, Huzhou University, Huzhou, Zhejiang 313000, China*

[k] *Key Laboratory of Marine Bio-resources Sustainable Utilization, South China Sea Institute of Oceanology, Chinese Academy of Sciences, Guangzhou, Guangdong 510301, China*

[l] *University of Sejong, Department of Environment, Energy and Geoinformatics, 98 Gunja-Dong, Guangjin-Gu, Seoul, South Korea*

[*]Corresponding author. E-mail: hailong.wang@fosu.edu.cn

Graphical abstract



Highlights

- Biochar's effect on pak choi growth in Cd-DEHP co-contaminated soils was tested.
- 2% pig biochar addition increased the yield of pak choi.
- Pig biochar improved nutrient phytoavailabilities more than wood biochar.
- Tested biochars enhanced soil urease, sucrase and catalase activities.
- Biochars had prominent influence on pak choi growth in low organic carbon soil.

Abstract

Reclamation of degraded soils such as those with low organic carbon content and soils co-contaminated with toxic elements and phthalic acid esters (PAEs) is of great concern. Little is known about the efficiency of plant- and animal-derived biochars for improving plant growth and the soil physicochemical and biological properties in these co-contaminated soils, particularly under low content of organic matter. Hence, a pot trial was carried out by growing pak choi (*Brassica chinensis* L.) to assess the influence of different doses (0, 0.5, 1, 2, and 4%) of animal (pig carcass) and wood

(*Platanus orientalis*) derived biochars on soil properties, nutrient availabilities, plant growth, and soil enzyme activities in two soils containing low (LOC) and high (HOC) organic carbon contents and co-contaminated with di-(2-ethylhexyl) phthalic acid (DEHP) and cadmium (Cd). Biochar applications significantly (P<0.05) improved pH, salinity, carbon content and cation exchange capacity of both soils. Addition of biochars significantly (P<0.05) increased the bioavailability and uptake of phosphorus and potassium in the plants in both soils with greater effects from pig biochar than wood biochar. Biochar additions also significantly (P<0.05) enhanced urease, sucrase, and catalase activities, but suppressed acid phosphatase activity in both soils. The impact of pig biochar was stronger on urease and acid phosphatase, while the wood biochar was more effective with sucrase, and catalase activities. The biomass yield of pak choi was significantly (P<0.05) increased after biochar addition to both soils, especially in 2% pig biochar treatment in the LOC soil. The positive response of soil enzymatic activities and plant growth for biochar addition to the Cd and DEHP co-contaminated soils indicate that both biochars could mitigate the risk of these pollutants and prove to be eco-friendly and low-cost amendments for reclaiming these degraded soils.

Keywords: Degraded land; nutrients availability; charcoal; soil biology; soil restoration.

1. Introduction

Industrialization, urbanization, effluent irrigation, uncontrolled disposal of wastes, agricultural plastic mulch abuse and other anthropogenic activities have resulted in unprecedented contamination of arable soils with heavy metal(loid)s (Qi et al., 2017; Bandara et al., 2019) and plasticizers, e.g., phthalic acid esters (PAEs) (Antoniadis et al., 2017; Zhao et al., 2019). Di-(2-ethylhexyl) phthalic acid (DEHP) as a typical PAE, and cadmium (Cd) as a typical heavy metal, have posed alarming

environmental and human health risks of these contaminants globally (Antoniadis et al., 2017; He et al., 2015; He et al., 2018; Bandara et al., 2019). They can be taken up by plants, decreasing the yield and quality of crops, and finally accumulated in human body through the food web, damaging the functions of human organs including endocrine and reproductive systems (Qin et al., 2018; Chen et al., 2019). Simultaneously, poor organic matter content of soils has been identified as a major reason for loosing soil quality and crop yield worldwide (Pulido-Fernández et al., 2013). Therefore, reclamation of degraded soils such as those co-contaminated with DEHP and heavy metal(loid)s, and soils with low organic matter content is of great importance. Achieving such reclamation via suitable low-cost amendments is an attractive option for soil restoration from both environmental quality and economic points of view (Yang et al., 2016; Palansooriya et al., 2019, 2020).

Numerous studies describing biochar as a suitable material for remediating organic pollutants (Zhang et al., 2013; Huang et al., 2018) and heavy metal(loid)s (Li et al., 2019a; Wu et al., 2017, 2019) in water (Li et al., 2019b; Mao et al., 2019) and soils (Shaheen et al., 2019; Yang et al., 2019) have been published. For instance, Abbas et al. (2017) found that the Cd concentration in wheat was decreased after rice straw biochar amendment. They claimed that the probable reason could be the reduction of Cd concentration in soil pore water for immediate crop uptake after biochar addition, and/or biochar facilitated the combined effects of Cd bioavailability reduction and soil organic matter improvement, as also suggested by Rizwan et al. (2016). In addition, biochar is of benefit to the improvement of soil structure (i.e., aggregate formation) (Quan et al., 2020) and fertility (Li et al., 2019c; Wei et al., 2019), and thereby promoting crop growth (Dong et al., 2015; Li et al., 2018; Purakayastha et al., 2019).

China generates around 20 million pig carcasses yearly, and this number continues to climb every

year (He et al., 2018). Additionally, urban green wastes such as tree branches have turned into a huge source of pollution and a hindrance to the benign development of ecological environment (Belyaeva and Haynes, 2010). Pyrolysis of pig carcasses and green wastes into biochar not only presents an efficient and environmentally friendly option for disposing these wastes (Yang et al., 2017) but also offers a tremendous scope for using the biochar for *in situ* remediation of soil contaminants while simultaneously improving soil productivity and crop yield.

In China, the area of vegetable cropping is second to grain production. Vegetables account for approximately 28.5% of the total diet in China, and pak choi (*Brassica chinensis* L.) is a typical widely-consumed leafy vegetable in daily life of the population (Yan et al., 2009). Wei et al. (2017) noted that the consumption of pak choi as a staple vegetable made a significant contribution to the estimated dietary intake of toxic metals such as Cd in Chinese population. As a consequence, it is of importance to reduce contaminant accumulation in pak choi and improve the crop yield and quality. It is well-accepted that soil enzymatic activity is sensitive to soil contaminants, accordingly considered to be a crucial parameter of soil health (He et al., 2019). Soil enzymes also have a critical influence on nutrient (e.g., K, N and P) cycling and subsequent uptake by plants (Sarkar et al., 2016; Nie et al., 2018). Nutrient phytoavailability affects plant growth directly while contamination stress can inhibit plant growth by posing toxic effects.

Till date, little information is documented on the efficiency of plant- and animal-derived biochars for affecting nutrient bioavailabilities, enzyme activities, and plant growth in DEHP-metal co-contaminated soils. We hypothesize that co-contamination of soils with Cd and DEHP may affect the soil microbial activities, enzyme activities, nutrient bioavailability, and plant growth, and these effects may differ based on the soil organic carbon content. To verify this hypothesis, we conducted a

pot-culture experiment using pak choi and two different soils treated with wood- and animal-derived biochars, i.e., pig carcasses and branches of *Platanus orientalis* Linn., to investigate the influence of biochars on the bioavailability of soil nutrients, enzyme activity, and the pak choi growth under the combined pollution of Cd and DEHP in soils containing low and high organic carbon contents.

2 Materials and methods

2.1 Soil and biochar collection, preparation, and characterization

The studied soils were sampled from two near-by fields (0-20 cm of topsoil) located in the southwest of Hangzhou City (30°24'N, 119°71'E), China. The first soil is rich in its total organic carbon content (HOC: 3.08%) and was used as farmland to cultivate vegetables nearly for twenty years. The second soil was left fallow for the same period, and thus was poor in its organic carbon content (LOC: 0.75%). Both soils were air-dried, crushed, and sieved (3-mm mesh). In order to obtain co-contaminated soils, the two soils were spiked with DEHP at 50 mg kg^{-1} soil and Cd at 1.0 mg kg^{-1}. The concentration of Cd^{2+} was referred to the Level 3 of the Environmental Quality Standards for soils GB 15618-1995, and DEHP concentration was chosen according to a previous research (He et al., 2016). The Cd/DEHP-spiked soils were mixed homogenously, air dried, and used for the pot experiment.

Pig biochar (PB) was produced by the pyrolysis of whole pig carcasses, and wood biochar (WB) was prepared by pyrolysing shredded branches (3-mm mesh) of *Platanus orientalis* Linn., at 650°C for 2 h. Both biochars were crushed and sieved (2-mm mesh) before mixing with the soils. The physicochemical properties of the studied biochars were determined using the methods described by Yang et al. (2016). The two biochars differed in many characteristics, such as ash content, cation

exchange capacity (CEC), available phosphorus, surface alkalinity and specific surface area. More details about the experimental soil and biochar properties, soil spiking procedure with DEHP and Cd, and soil preparation and characterization are included in Supporting Information (Appendix A) and published in Chen et al. (2019).

2.2 Pot trial

The pot trial was carried out in a greenhouse located in Zhejiang A&F University, Zhejiang Province, China, at temperature between 25 to 33^{o}C. Each ceramic pot (20 cm diameter, 19 cm height) was filled with either 3 kg of the Cd-DEHP contaminated LOC or HOC soil. Then the pig biochar and wood biochar were applied to the Cd-DEHP contaminated soils in the ceramic pots at five doses (i.e., 0, 0.5, 1, 2 and 4%, w/w) and mixed well. In total, eighteen treatments (including controls) were set in this trial and every treatment repeated in four replicates. The LOC and HOC control soils did not receive any dose of biochar.

All pots were fertilized with KH_2PO_4 and urea according to a basal dose of K_2O 0.2 g·kg^{-1}, P_2O_5 0.32 g·kg^{-1}, N 0.25 g kg^{-1} recommended for pak choi (He et al., 2016). Treatments were arranged in a complete randomized block design. The soil was maintained at 70% of the field water holding capacity for an initial period of 30 days to equilibrate the spiked Cd and DEHP into the soil. After the equilibration, ten pak choi seeds were sown at equal spacing in each ceramic pot on 10 July 2017. After about fifteen days, five strongest seedlings were kept after thinning out the rest. Watering with deionized water (2-3 times per week) was performed during the growth period to maintain the soil moisture status at the field capacity. After maturity (50 days), the matured pak choi shoots were harvested from the pots. The plants were rinsed with deionized water to get rid of the soil particles.

156 The fresh plant shoots were oven-dried at 105ºC for 0.5 h and subsequently oven-dried at 65ºC until a
157 constant weight was achieved. Dried plant shoots were crushed and sieved (0.25-mm mesh) before
158 chemical analysis. After plant harvest, the soils in each pot were collected, homogenized and air-dried.
159 Sampled soils were then ground to 2-mm and 0.25-mm fractions for further chemical analysis.
160
161 **2.3 Soil analysis**
162 The dry and ground soils were analyzed for pH, electrical conductivity (EC), organic carbon
163 content (OC), cation exchange capacity (CEC) and particle size distribution according to the methods
164 described by Lu (2000). Soil available potassium (K) was extracted using ammonium acetate, and
165 analyzed by a flame photometer (FP640, Xinyi Instrument, China) (Lu, 2000). The concentration of
166 available nitrogen (N) was extracted using a micro-diffusion technique after alkaline-hydrolysis
167 method (Lu, 2000). The available phosphorus (P) was extracted using sodium bicarbonate ($NaHCO_3$)
168 and measured by spectrophotometric method (UVA 132122, Thermo Electron Corporation, England)
169 at 700 nm wavelength (Lu, 2000). The total Cd content of the soils was determined by digesting the
170 soils with HF-$HClO_4$-HNO_3 (Carignan and Tessier, 1988). Potentially available Cd was extracted by
171 diethylenetriaminepentaacetate acid (DTPA) (Lu, 2000). Cadmium was analyzed using inductively
172 coupled plasma optical emission spectroscopy (ICP-OES Optima 2000, PerkinElmer Co., USA). The
173 DEHP was extracted and analyzed as per He et al. (2016). More details about the determination
174 methods of Cd and DEHP concentrations in soil are provided in the Supporting Information
175 (Appendix A).
176

177 **2.4 Soil enzyme activities**

178 The activities of urease, acid phosphatase, sucrase, and catalase were determined according to
179 Dick et al. (1996). The urease activity was expressed as the mass of NH_3-N released per gram of dry
180 soil after 24-hour incubation with urea solution at 37°C and determined by spectrophotometric method
181 at 578 nm wavelength. The acid phosphatase activity was expressed as the mass of phenol released
182 per gram of dry soil after 24-hour incubation with a p-nitrophenyl phosphate substrate at 37°C and
183 determined by spectrophotometric method at 660 nm wavelength. The sucrase activity was expressed
184 as mass of glucose released per gram of dry soil after 24-hour incubation with glucose solution at
185 37°C and determined by spectrophotometric method at 508 nm wavelength. The catalase activity was
186 measured by titrating the residual hydrogen peroxide (H_2O_2) added after 20 minutes of soil exposure
187 with 0.1 M potassium permanganate ($KMnO_4$). The catalase activity was expressed as the volume of
188 0.1 M $KMnO_4$ used per gram dry soil per minute (Dick et al., 1996).

189

190 **2.5 Plant biomass and analysis of nutrients in plants**

191 The dry weight of the plant shoots was recorded, and the samples were kept for further analysis.
192 The nitrogen (N) concentration was measured using an elemental analyzer (Flash EA1112, Thermo
193 Finnigan, Italy).

194 Plant shoots were digested with nitric acid (HNO_3), and the P, K, and Cd concentrations were
195 determined. The P concentration was quantified by spectrophotometric method (UVA 132122,
196 Thermo Electron Corporation, England) at 700 nm (Lu, 2000). The concentration of K was
197 determined by a flame photometer (FP640, Xinyi Instrument, China), the concentration of Cd was
198 determined with ICP-OES (Optima 2000, PerkinElmer Co., USA) (Lu, 2000).

199

200 **2.6 Data analysis**

201 Data analysis was performed with the statistical package SPSS 17.0. Variability of data was
202 expressed in terms of standard deviation of four replicates. Analysis of variance (ANOVA) was used
203 to assess differences between treatments, and $P<0.05$ was supposed to be statistically significant.
204 Pearson's correlation analysis with a significance level of $P<0.01$ was performed to identify the
205 correlation between variables.

206

207 **3 Results and discussion**

208 **3.1 Biochar-induced changes in soil pH, salinity, CEC, and organic carbon**

209 Soil pH significantly ($P<0.05$) increased after application of the wood and pig biochars in both
210 the LOC and HOC soils, and the impact of biochars was based on the applied dosage (Fig. 1A). The
211 increase of soil pH might be due to the high pH of biochars (9.5 for wood biochar and 10.0 for pig
212 biochar; Table S2). We assume that when these alkaline biochars were applied into the soil, the alkali
213 salts might be released, and thus increase the soil pH (Martinsen et al., 2015). Application of pig
214 biochar made a greater impact on soil pH than wood biochar, which might be due to the higher pH,
215 ash content, and surface alkalinity of the pig biochar (Appendix A; Table S2). Biochar addition also
216 improved the status of the water-soluble salts, and thus increased the soil salinity, particularly in the
217 HOC soil (Fig. 1 B), which might be due to the high mineral contents of the biochars (Fig. S1).
218 However, the values of EC were still less than 0.3 dS m^{-1}, which means that the biochar treated soils
219 would not suffer from high salinity.

220 Applications of 4% pig and wood biochars were effective in increasing the CEC in both soils

(Fig. 1C). The increase of soil CEC after addition of biochars might be explained by the high surface alkalinity and ash content of the biochars as indicated in Table S2. Wood biochar addition was more efficient in increasing the organic carbon content of soil than pig biochar (Fig. 1D), which can be explained by its higher content of carbon than pig biochar (Table S2). For instance, the highest soil organic carbon contents were noticed at 4% wood biochar treatments, which increased by 5.4 folds in the LOC soil and 0.7 folds in the HOC soil, as compared to the control.

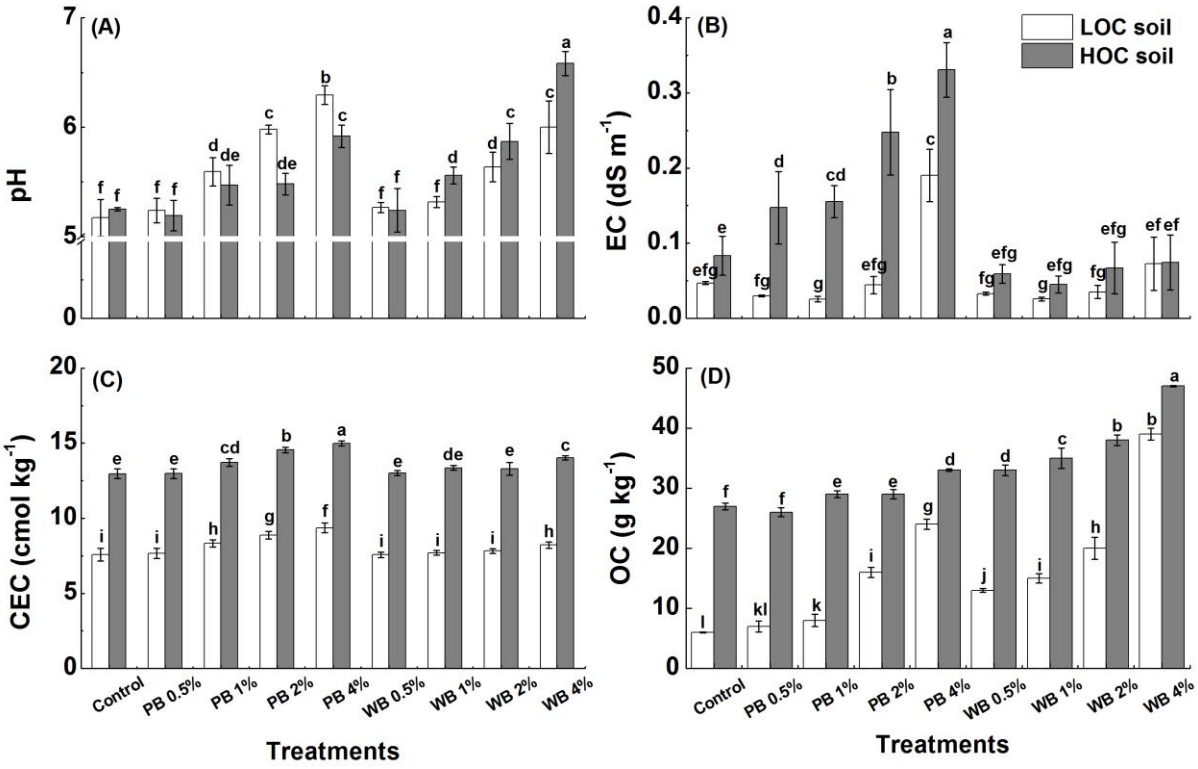


Fig. 1. Effect of biochar treatments on the pH (A), electrical conductivity (EC) (B), cation exchange capacity (CEC) (C), and organic carbon (OC) (D) in low organic carbon (LOC) soil and high organic carbon (HOC) soil. Control: untreated soil contaminated by Cd-DEHP; PB: pig biochar; WB: wood biochar. Error bars are standard deviation of the means (n=4). Different lower-case letters above the columns indicate significant difference between treatments (P<0.05).

3.2 Impact of biochars on the bioavailability and uptake of N, P, and K

The pig biochar addition caused a more profound impact than wood biochar in increasing the

237 bioavailability and uptake of K and P in soils (Fig. 2A and 2B). The maximum values of available K
238 corresponded to 4% pig biochar treatment, with up to 4.1-fold increase in the LOC soil and up to
239 2.1-fold increase in the HOC soil. In addition, the concentrations of available P in pig
240 biochar-amended LOC soil increased by 1.0-3.5 folds, and in HOC soil, it increased by 0.4-0.8 folds.
241 Simultaneously, the concentrations of K and P in plants also significantly (P<0.05) increased as the
242 pig biochar application dosage increased (Fig. 2D and 2E). Compared to the controls, significant
243 increases of available K in soils were also noticed after wood biochar addition, which increased by
244 0.7-1.8 and 0.3-0.5 folds in the LOC and HOC soils, respectively. However, wood biochar amendment
245 showed a non-significant (P>0.05) effect on the bioavailability of P neither in LOC nor HOC soil.
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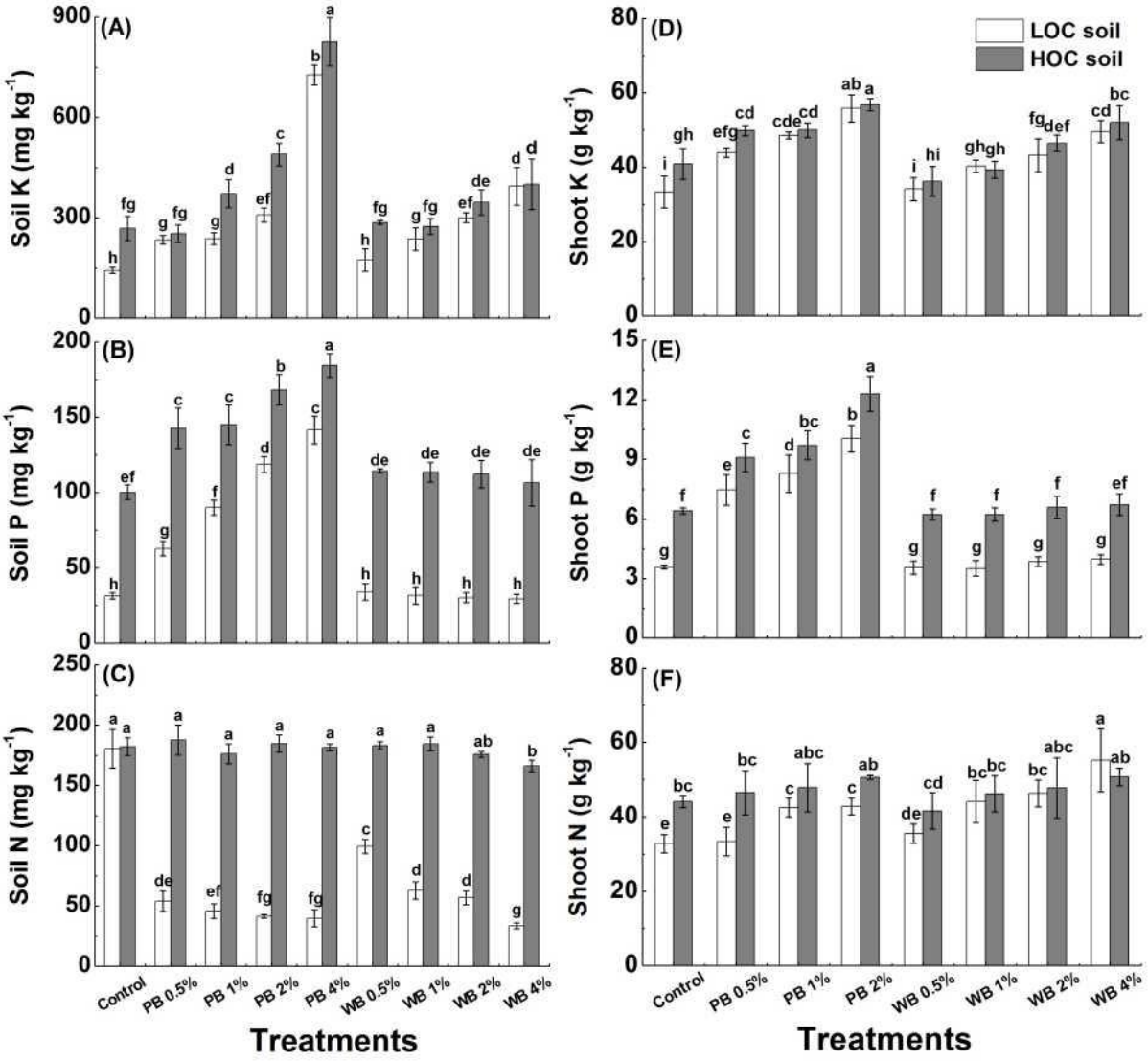

Fig. 2. Effect of biochar treatments on the available K (A), P (B) and N (C) in the low organic carbon (LOC) soil and high organic carbon (HOC) soil, and the uptake of K (D), P (E) and N (F) in plant shoots. Control: untreated soil contaminated by Cd-DEHP; PB: pig biochar; WB: wood biochar. Error bars are standard deviation of the means (n=4). Different lower-case letters above the columns indicate significant difference between treatments (P<0.05).

Increasing the bioavailability and uptake of K and P in the pig biochar-treated soils might be due to the higher contents of P and K in the pig biochar than wood biochar (Table S2). Improving the availability of P and K in biochar treated soils agrees with previous studies (e.g., Yang et al., 2016; Purakayastha et al., 2019). The biochar-induced improvement of soil pH and CEC could also be

another reason for improving the status and availability of P and K in the pig biochar-treated soil (DeLuca et al., 2009; Haefele et al., 2011). We also assume that the biochar-induced enhancement of soil microbial activities could be a reason for increasing the bioavailability of P and K in the wood biochar-treated soils. Our hypothesis was supported by the improvement of soil enzyme activities in the biochar treated soils, as shown in Fig. 3, and will be discussed in section 3.3. In this respect, Wardle et al., (2008) and Gul et al. (2015) indicated that biochar application might promote the growth and activity of soil microorganisms via improving the soil structure (e.g., facilitating soil temperature, moisture and aeration), and functioning as a carbon source, and therefore enhance P and K mineralization. In addition, we also assume that the mitigated biotoxicity of Cd and DEHP could enhance P and K uptake. Previous studies (Sun et al., 2018; Chen et al., 2019) found that the existence of Cd and DEHP would damage the cell membranes in major plants, and the destruction of cell membranes seriously affected the absorption of nutrient elements by blocking the transmembrane transport. Therefore, application of biochars might indirectly promote the absorption of P and K by plants via alleviating the stress of contaminants in soils.

The biochar impact on soil available N content was stronger in the LOC soil than the HOC soil (Fig. 2C). The available N concentration in the LOC soil significantly (P<0.05) decreased with the addition of both biochars. However, the shoot N concentration increased after both biochars' addition (Fig. 2F). We hypothesize that the decrease of N availability in the LOC soil after the addition of both biochars could be ascribed to their specific properties (i.e., high porosity, specific surface area and CEC), which might increase the sorption of NO_3^- (pore filling) and NH_4^+ (cation exchange), as also reported by other studies (e.g., Olmo et al., 2016; Purakayastha et al., 2019). In addition, the C-rich biochars used in the current study would increase the C/N ratio of biochar-amended soil, which might

inhibit the mineralization rate of soil organic N by reducing the activities of microorganisms, and thereby decrease the N availability, as the similar interpretations were previously reported by Haefele et al. (2011). The increase of N concentration in plants might not be due to the extra N provided by biochar, because most of N would be non-bioavailable in biochar pyrolyzed at a temperature higher than 500^{o}C (650^{o}C in this study) (Zheng et al., 2013; Lu et al., 2014). In regards to the increase of shoot N concentration after biochar application increased, we assume that it could be attributed to the improvement of N utilization efficiency after biochar application into the soil, according to the results reported by Zheng et al., (2013) and Purakayastha et al. (2019).

Additionally, the influence of biochar application on the availability of K, P and N of LOC soils was more noticeable than that of HOC soils, which suggested that the soil organic carbon content had a strong association with the effectiveness of biochar application on impacting the soil fertility. Yang et al. (2016) demonstrated that the higher organic carbon content increased the soil buffering capacity. Thus, in our present study, it is interpretable that biochar application had more advantages in improving the physicochemical properties and nutrient availabilities of the LOC soil than that of HOC soil.

3.3 Impact of biochars on enzyme activities

Soil enzyme activities, as biological/biochemical indicators of soil quality, are closely related to the behavior of soil microorganisms, and could be affected by soil contamination (Bandara et al., 2019; He et al., 2019). As shown in Fig. 3, application of biochars had positive effect on the activities of urease, sucrase, and catalase, and the effectiveness differed based on biochar type and dose, and soil types. Compared to the untreated soils, the urease activity of the LOC and HOC soils treated with all doses of pig biochar increased by 19.0-133.6% and 58.3-213.0%, respectively (Fig. 3A). In the case

of wood biochar treatments, only the 4% dose led to a significant (P<0.05) increase in urease activity in LOC soil; however, in the HOC soil, the urease activity was significantly increased for all biochar treatments as compared to the control (Fig. 3A). However, application of 4% wood biochar had a greater impact on enhancing the activities of sucrase and catalase than urease. The maximum values of sucrase activity were noticed at 4% wood biochar treatment, with up to 12.5-fold increase in the LOC soil, and 4.6-fold increase in the HOC soil, as compared to the control soil (Fig. 3C). The wood biochar was more effective (increased by 85.8-150.8% in LOC soils, and 27.2-65.2% in HOC soils) than pig biochar application (increased by 35.0-140.5% in LOC soils, and 19.0-60.5% in HOC soils) in increasing the catalase activity in soils (Fig. 3D).

We hypothesize that the enhancement of urease, sucrase, and catalase activities of soil with biochar application might be due to the high mineral and nutrient contents, porosity and surface area of the added biochars (Table S2; Fig. S1), which provided a habitat for microorganisms with ample aeration, water, and nutrients, which might be a reason for improving the growth and reproduction of soil microorganisms, as reported by Gul et al. (2015) and Bandara et al. (2019), and thereby promoting soil enzymatic activities.

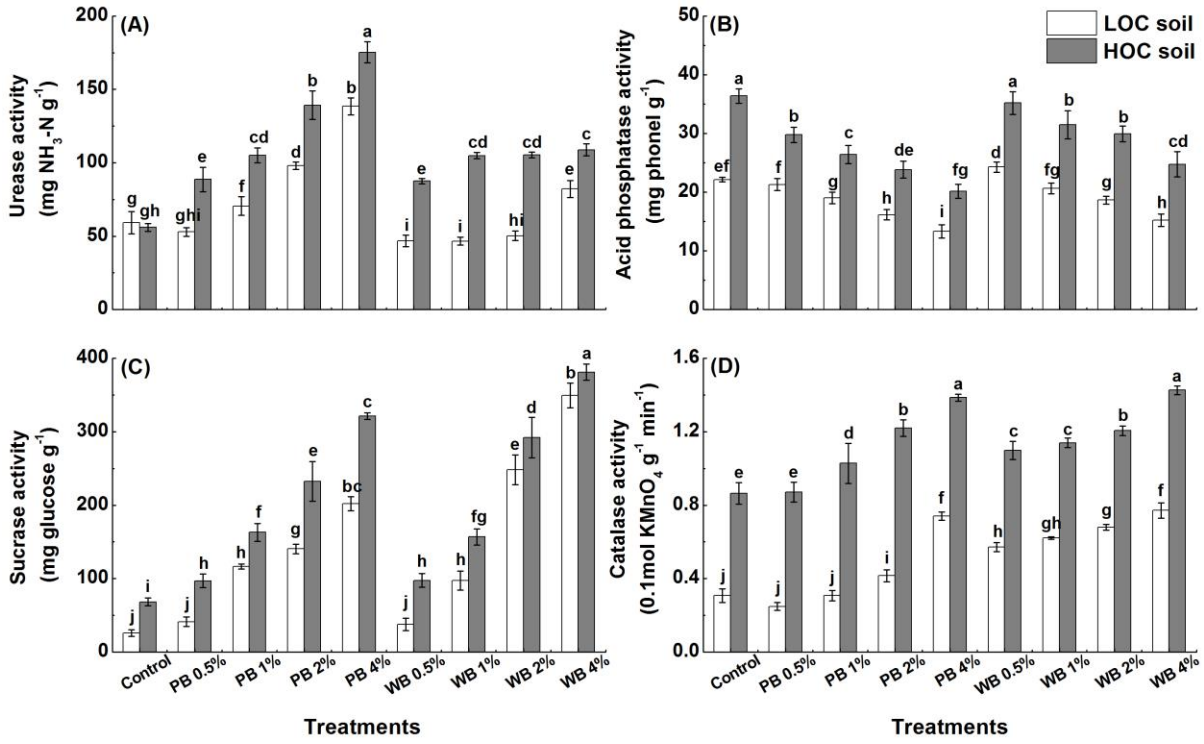

Fig. 3. Effect of biochar treatments on the activities of urease (A), acid phosphatase (B), sucrase (C) and catalase (D) in low organic carbon (LOC) soil and high organic carbon (HOC) soil. Control: untreated soil contaminated by Cd-DEHP; PB: pig biochar; WB: wood biochar. Error bars are standard deviation of the means (n=4). Different lower-case letters above the columns indicate significant difference between treatments (P<0.05).

Toxic metals, such as Cd ions, might deactivate the enzyme proteins, and thus inhibit soil enzymatic activities (Tan et al., 2018). Also, DEHP might affect the production of enzymes by causing dysfunction in the structure of cell membrane (Chen et al., 2019). Improving the activities of urease, sucrase, and catalase in the biochar treated soils as compared to the untreated soils indicated that both biochars mitigated the negative impact of Cd and DEHP on these enzyme activities in the contaminated soils. In our previous study (Chen et al., 2019), both the wood and pig biochars, particularly pig biochar, were able to reduce the bioavailability of Cd and DEHP in both soils. Therefore, we assume that the biochar-induced reduction of Cd and DEHP toxicity in biochar-amended soils might promote the soil enzyme activities in these soils as compared to the

335 untreated ones. Pearson's correlation analysis in the present study provided a proof that the urease
336 activity negatively correlated to the concentration of extractable Cd ($r = -0.489$, $P<0.01$, $n = 72$), and
337 the catalase activity negatively correlated to the DEHP concentration in soil ($r = -0.527$, $P<0.01$, $n =$
338 72). These results indicated that biochar application was able to reduce the Cd and DEHP bio-toxicity
339 through adsorption/immobilization of those contaminants onto biochar (Qin et al., 2018), as suggested
340 by the improvement of most of the enzymatic activities examined in this study.

341 On other hand, the acid phosphatase activity decreased significantly ($P<0.05$) in the biochar
342 treated soil as compared to the control (Fig. 3 B). Pig biochar addition decreased (14.1-39.8% in LOC
343 soil and 18.2-44.7% in HOC soil) the acid phosphatase activity more than wood biochar application
344 (15.7-31.3% in LOC soil and 13.5-32.1% in HOC soil), in comparison to untreated soils (Fig. 3B). We
345 hypothesize that the reduction of acid phosphatase activity in the biochar treated soils could be
346 interpreted by the associated increase of soil pH, as also indicated by Chen et al. (2013) and Yang et al.
347 (2016). Wang et al. (2018) reported that the acid phosphatase activity depended on soil microbial
348 activities and soil pH. The optimum pH of acid phosphate activity is pH=4.0-5.0 (Wang et al., 2018);
349 however, our soil pH increased to 6.5 after biochar addition, which might cause an inhibitory effect on
350 the acid phosphatase activity. A significant negative correlation between acid phosphatase activity and
351 soil pH was observed in this study ($r = -0.434$, $P<0.01$, $n = 72$), which also presented an evidence for
352 our hypothesis.

353 The acid phosphatase activity relates to P transformation and cycling in the soil, and the urease is
354 a crucial factor in soil N mineralization (Yang et al., 2016; Wang et al., 2018). Pig biochar had a more
355 profound influence on soil N and P availability than wood biochar. Therefore, pig biochar amendment
356 had greater effect on the urease and acid phosphatase activities in soil, and the reason might be the

higher N and P contents, CEC, surface alkalinity of pig biochar than wood biochar. Sucrase and catalase activities depended on soil organic carbon content, and therefore, the higher C content in wood biochar than pig biochar might enhance sucrase and catalase activities in the biochar-amended soils. Further research should be performed to determine the reasons for different response of biochar-amended soil enzyme activities to the LOC and HOC soils.

3.4 Impact of biochars on plant growth

Given that the seeds of pak choi in 4% pig biochar treatments did not germinate normally, we eliminated the plant data from that treatment in the statistical analysis. The fact that pak choi did not germinate well in the 4% pig biochar treatments in the pot trial suggested that high pig biochar dosage (4%) inhibited plant growth, as observed also by Schmidt et al. (2014) and Khan et al. (2015). Schmidt et al. (2014) reported that high biochar dosage might cause nutrient immobilization in soils, particularly the dissolved organic carbon and mineral N, which consequently would restrict plant growth. Another possible reason was that biochar application increased the available NH^{4+}-N concentration in the soil to a level which led to a stress condition for the plant (Khan et al., 2015).

Fig. 4 showed that all treatments (except in the 0.5% wood biochar treatment) significantly (P<0.05) increased the dry weight of plant shoots, as compared to the control. The highest dry weight of plant shoot was observed in 2% pig biochar treatment, which was 10.1 and 0.5 folds higher than the control in the LOC and HOC soil, respectively. In both soils, pig biochar amendment was more effective in enhancing the shoot dry weight than wood biochar amendment. In addition, the impact of biochar on plant dry weight in the LOC soil was stronger than that in the HOC soil. The increase of dry weight biomass of plants in the biochar treated soils can be explained by the associated increase of soil nutrient availabilities and improved soil physical and chemical properties, as discussed in the

previous sections and reported by other studies (e.g., Haefele et al., 2011; El-Naggar et al., 2018; Purakayastha et al., 2019). The pig biochar had higher nutrient contents than wood biochar, and thus pig biochar showed a greater effect on plant growth than wood biochar.

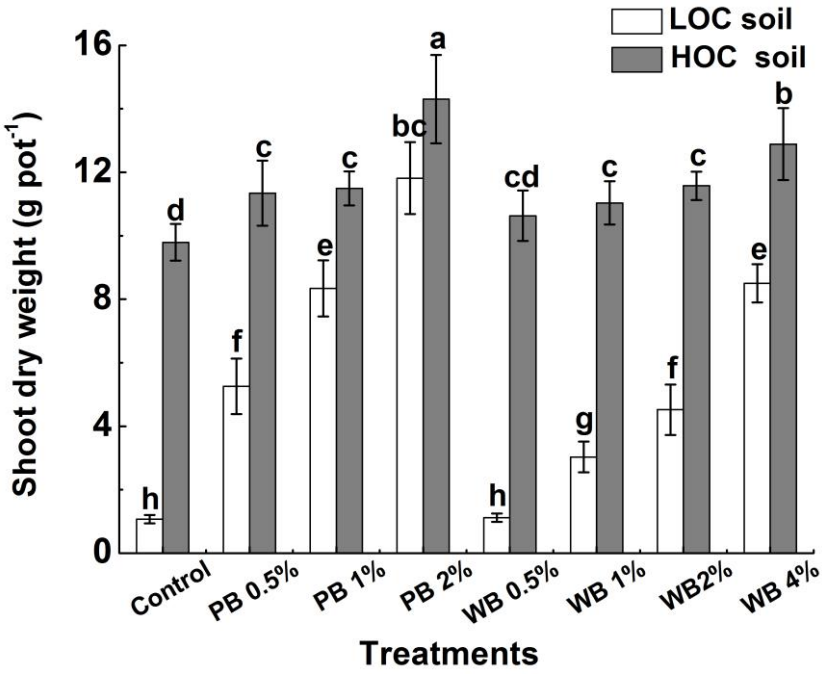

Fig. 4. Effect of biochar treatments on plant shoots dry weight. LOC: low organic carbon content; HOC: high organic carbon content; Control: untreated soil contaminated by Cd-DEHP; PB: pig biochar; WB: wood biochar. Error bars are standard deviation of the means (n=4). Different lower-case letters above the columns indicate significant difference between treatments (P<0.05).

Cadmium and DEHP can negatively affect the plant growth in contaminated soils. In our studied soils, the relationships between plant growth and Cd and DEHP concentrations in the plant were negative (Fig. S2). Improvement of plant biomass in the biochar treated soils as compared to the untreated soils indicated that both biochars, particularly 2% of pig biochar, mitigated the negative impact of Cd and DEHP on the plant growth in these co-contaminated soils. This positive impact agrees with Lu et al. (2014) who demonstrated that the addition of bamboo and rice straw biochar

increased the shoot biomass of *S. plumbizincicola* in metal contaminated soil through improving the soil pH. In our experimental soils, the increased soil alkalinity (Fig. 1A) could be a reasonable factor for immobilizing Cd and DEHP in the soil and reducing their uptake by the plants (Chen et al., 2019), and thus promoting the crop productivity after biochar application to the acidic soil.

In our previous study (Chen et al., 2019), we found that biochar application decreased Cd and DEHP bioaccumulation in pak choi shoot, and the pig biochar application was more efficient in comparison with wood biochar. The effect of biochars on the shoot dry weight was more prominent in the LOC soil than in the HOC soil, which agrees with reports by Haefele et al. (2011) and Zhang et al. (2012) who concluded that biochar produced from crop straw increased rice yield more significantly in barren soils than fertile soils.

4. Conclusions

Our study provided promising information of using animal carcass- and wood-derived biochars for reclamation of degraded soils, such as Cd-DEHP co-contaminated soils and soils with low organic matter content. Both biochars improved the soil properties (e.g., pH, carbon content, and CEC), increased the bioavailability of P and K in soils and the uptake of P, K, and N by pak choi, and improved the activities of urease, sucrase, and catalase activities. Both biochars, particularly 2% pig biochar, increased the plant biomass, especially in the LOC soil. The positive response of soil enzyme activities and plant growth due to biochar addition in the Cd-DEHP co-contaminated soils indicated that these two biochars could mitigate the risk of Cd and DEHP in soils and improve the soil quality. Pig biochar had higher pH, ash content, surface alkalinity, CEC, and nutrient contents than wood biochar; therefore, the former showed more potential to improve soil properties, nutrient availability, and urease activities, and thereby enhanced the crop yield more than wood biochar. This study thus

offers a preliminary understanding of employing pig biochar as an emerging eco-friendly biosorbent for improving soil fertility and crop quality in heavy metal-PAE co-contaminated soils, as well as a cost-effective and applicable fertilizer in degraded soils.

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Appendix A. Supplementary data

Supplementary data to this article can be found online.

References

Abbas, T., Rizwan, M., Ali, S., Rehman, M.Z., Qayyum, M.F., Abbas, F., Hannan, F., Rinklebe, J., Ok, Y.S., 2017. Effect of biochar on cadmium bioavailability and uptake in wheat (*Triticum aestivum* L.) grown in a soil with aged contamination. Ecotox. Environ. Safe. 140, 37-47.

Antoniadis, V., Levizou, E., Shaheen, S.M., Yong, S.O., Sebastian, A., Baum, C., Prasad, M.N.V., Wenzel, W.W., Rinklebe, J., 2017. Trace elements in the soil-plant interface: phytoavailability, translocation, and phytoremediatione: A review. Earth Sci. Rev. 171, 621-645.

Bandara, T., Franks, A., Xu, J., Bolan, N., Wang, H., Tang, C., 2019. Chemical and biological immobilization mechanisms of potentially toxic elements in biochar-amended soils. Crit. Rev. Environ. Sci. Technol. (in press).

Belyaeva, O.N., Haynes, R.J., 2010. A comparison of the properties of manufactured soils produced from composting municipal green waste alone or with poultry manure or grease trap/septage waste[J]. Biol Fert Soils. 46(3), 271-281.

Carignan, R., Tessier, A., 1988. The co-diagenesis of sulfur and iron in acid lake sediments of

445 southwestern Québec. Geochim Cosmochim Ac. 52, 1179-1188.
446 Chen, H., Yang, X., Gielen, G., Mandal, S., Xu, S., Guo, J., Shaheen, S.M., Rinklebe, J., Che, L.,
447 Wang, H., 2019. Effect of biochars on the bioavailability of cadmium and di-(2-ethylhexyl)
448 phthalate to *Brassica chinensis* L. in contaminated soils. Sci. Total Environ. 678, 43-52.
449 Chen, J., Liu, X., Zheng, J., Zhang, B., Lu, H., Chi, Z., Pan, G., Li, L., Zheng, J., Zhang, X., Wang, J.,
450 Yu, X., 2013. Biochar soil amendment increased bacterial but decreased fungal gene abundance
451 with shifts in community structure in a slightly acid rice paddy from Southwest China. Appl. Soil
452 Ecol. 71, 33-44.
453 DeLuca, T.H., MacKenzie, M.D., Gundale, M.J., 2009. Biochar effects on soil nutrient
454 transformations. In: Lehman, J., Joseph, S. (Eds.), Biochar for Environmental Management.
455 Earthscan, London, UK, p. 251-270.
456 Dick, R.P., Breakwell, D.P., Turco, R.F., Doran, J.W., Jones, A.J., 1996. Soil enzyme activities and
457 biodiversity measurements as integrative microbiological indicators.
458 Dong, D., Feng, Q., McGrouther, K., Yang, M., Wang, H., Wu, W., 2015. Effects of biochar
459 amendment on rice growth and nitrogen retention in a waterlogged paddy field. J. Soils Sediments
460 15, 153-162.
461 El-Naggar, A., Lee, S.S., Awad, Y.M., Yang, X., Ryu, C., Rizwan, M., Rinklebe, J., Tsang, D.C., Ok,
462 Y.S., 2018. Influence of soil properties and feedstocks on biochar potential for carbon
463 mineralization and improvement of infertile soils. Geoderma 332, 100-108.
464 Gul, S., Whalen, J.K., Thomas, B.W., Sachdeva, V., Deng, H., 2015. Physico-chemical properties and
465 microbial responses in biochar-amended soils: Mechanisms and future directions[J]. Agric.
466 Ecosyst. Environ. 206: 46-59.
467 Haefele, S.M., Konboon, Y., Wongboon, W., Amarante, S., Maarifat, A.A., Pfeiffer, E.M., Knoblauch,
468 C., 2011. Effects and fate of biochar from rice residues in rice-based systems. Field Crop Res.
469 121(3), 430-440.
470 He, D., Cui, J., Gao, M., Wang, W., Zhou, J., Yang, J., Wang, J., Li, Y., Jiang, C., Peng, Y., 2019.
471 Effects of soil amendments applied on cadmium availability, soil enzyme activity, and plant uptake
472 in contaminated purple soil. Sci. Total Environ. 654, 1364-1371.
473 He, L., Fan, S., Müller, K., Hu, G., Huang, H., Zhang, X., Lin, X., Che, L., Wang, H., 2016. Biochar
474 reduces the bioaccessibility of di-(2-ethylhexyl) phthalate in soil. Chemosphere 142, 24-27.

He, L., Fan, S., Müller, K., Wang, H., Che, L., Xu., S., Song, Z., Yuan, G., Rinklebe, J., Tsang, D.C.W., Ok, Y.S., Bolan, N., 2018. Comparative analysis biochar and compost-induced degradation of di-(2-ethylhexyl) phthalate in soils. Sci. Total Environ. 625, 987-993.

He, L., Gielen, G., Bolan, N.S., Zhang, X., Qin, H., Huang, H., Wang, H., 2015. Contamination and remediation of phthalic acid esters in agricultural soils in China: a review. Agron. Sustain. Dev. 35, 519-534.

Huang, P., Ge, C., Feng, D., Yu, H., Luo, J., Li, J., Strong, P.J., Sarmah, A.K., Bolan, N.S., Wang, H., 2018. Effects of metal ions and pH on ofloxacin sorption to cassava residue-derived biochar. Sci. Total Environ. 616-617, 1384-1391.

Khan, S., Waqas, M., Ding, F., Shamshad, I., Arp, H.P.H., Li, G., 2015. The influence of various biochars on the bioaccessibility andbioaccumulation of PAHs and potentially toxic elements toturnips (*Brassica rapa L.*). J. Hazard. Mater. 300, 243-253

Li, J., Wang, S., Zheng, L., Chen, D., Wu, Z., Xie, Y., Wu, W., Niazi, N.K., Ok, Y.S., Rinklebe, J., Wang, H., 2019a. Sorption of lead in soil amended with coconut fiber biochar: Geochemical and spectroscopic investigations. Geoderma 350, 52-60.

Li, J., Zheng, L., Wang, S.-L., Wu, Z., Wu, W., Niazi, N.K., Shaheen, S.M., Rinklebe, J., Bolan, N., Ok, Y.S., Wang, H., 2019b. Sorption mechanisms of lead on silicon-rich biochar in aqueous solution: Spectroscopic investigation. Sci. Total Environ. 672, 572-582.

Li, Y., Hu, S., Chen, J., Müller, K., Li, Y., Fu, W., Lin, Z., Wang, H., 2018. Effects of biochar application in forest ecosystems on soil properties and greenhouse gas emissions: a review. J. Soils Sediments 18, 546-563.

Li, Z., Song, Z., Singh, B.P., Wang, H., 2019c. The impact of crop residue biochars on silicon and nutrient cycles in croplands. Sci. Total Environ. 659, 673-680.

Lu, K., Yang, X., Shen, J., Robinson, B., Huang, H., Liu, D., Bolan, N., Pei, J., Wang, H., 2014. Effect of bamboo and rice straw biochars on the bioaccessibility of Cd, Cu, Pb and Zn to *Sedum plumbizincicola*. Agric. Ecosyst. Environ. 191, 124-132.

Lu, K., Yang, X., Gielen, G., Bolan, N., Ok, Y.S., Niazi, N.K., Xu, S., Yuan, G., Chen, X., Zhang, X., Liu, D., Song, Z., Liu, X., Wang, H., 2017. Effect of bamboo and rice straw biochars on the mobility and redistribution of heavy metals (Cd, Cu, Pb and Zn) in contaminated soil. J. Environ. Manage. 186, 285-292.

505 Lu, R.K., 2000. Analytical Methods for Soil Agrochemistry. Chinese Agricultural Science and
506 Technology Publishing House, (in Chinese), Beijing.
507 Mao, Q., Zhou, Y., Yang, Y., Zhang, J., Liang, L., Wang, H., Luo, S., Luo, L., Jeyakumar, P., Ok, Y.S.,
508 Rizwan, M., 2019. Experimental and theoretical aspects of biochar-supported nanoscale
509 zero-valent iron activating H_2O_2 for ciprofloxacin removal from aqueous solution. J. Hazard.
510 Mater. 380, 120848.
511 Martinsen, V., Alling, V., Nurida, N.L., Mulder, J., Hale, S.E., Ritz, C., Rutherford, D.W., Heikens, A.,
512 Breedveld, G.D., Cornelissen, G., 2015. pH effects of the addition of three biochars to acidic
513 Indonesian mineral soils. Soil Sci. Plant Nutr. 61, 821-834.
514 Nie, C., Yang, X., Niazi, N.K., Xu, X., Wen, Y., Rinklebe, J., Ok, Y.S., Xu S., Wang, H., 2018. Impact
515 of sugarcane bagasse-derived biochar on heavy metal availability and microbial activity: A field
516 study. Chemosphere 200, 274-282.
517 Olmo, M., Villar, R., Salazar, P., Alburquerque, J.A., 2016. Changes in soil nutrient availability
518 explain biochar's impact on wheat root development. Plant Soil 399 (1-2), 333-343.
519 Palansooriya, K.N., Shaheen, S.M., Chen, S.S., Tsang, D.C.W., Hashimoto, Y., Hou, D., Bolan, N.,
520 Rinklebe, J., Ok, Y.S., 2020. Soil amendments for immobilization of potentially toxic elements in
521 contaminated soils: A critical review. Environment International.
522 doi.org/10.1016/j.envint.2019.105046
523 Palansooriya, K.N., Wong, J.T.F., Hashimoto, Y., Huang, L., Rinklebe, J., Chang, S.X., Bolan, N.,
524 Wang, H., Ok, Y.S., 2019. Response of microbial communities to biochar-amended soils: a critical
525 review. Biochar 1, 3-22.
526 Pulido-Fernández, M., Schnabel, S., Lavado-Contador, J.F., Miralles Mellado, I., Ortega Pérez, R.,
527 2013. Soil organic matter of Iberian open woodland rangelands as influenced by vegetation cover
528 and land management. Catena 109, 13–24.
529 Purakayastha, T.J., Bera, T., Bhaduri, D., Sarkar, B., Mandal, S., Wade, P., Kumari, S., Biswas, S.,
530 Menon, M., Pathak, H., Tsang, D.C.W., 2019. A review on biochar modulated soil condition
531 improvements and nutrient dynamics concerning crop yields: Pathways to climate change
532 mitigation and global food security. Chemosphere 227, 345-365.
533 Qi, F., Kuppusamy, S., Naidu, R., Bolan, N.S., Ok, Y.S., Lamb, D., Li, Y., Yu, L., Semple, K.T., Wang,
534 H., 2017. Pyrogenic carbon and its role in contaminant immobilization in soils. Crit. Rev. Environ.

Sci. Technol. 47, 795-876.

Qin, P., Wang, H., Yang, X., He, L., Müller, K., Shaheen, S.M., Xu, S., Rinklebe, J., Tsang, D.C.W., Ok, Y.S., 2018. Bamboo- and pig-derived biochars reduce leaching losses of dibutyl phthalate, cadmium, and lead from co-contaminated soils. Chemosphere 198, 450-459.

Quan, G., Fan, Q., Sun, J., Cui, L., Wang, H., Gao, B., Yan, J., 2020. Characteristics of organo-mineral complexes in contaminated soils with long-term biochar application. J. Hazard. Mater. 384, 121265.

Rizwan, M., Ali, S., Qayyum, M.F., Ibrahim, M., Rehman, M.Z., Abbas, T., OK, Y.S., 2016. Mechanisms of biochar-mediated alleviation of toxicity of trace elements in plants: a critical review. Environ. Sci. Pollut. Res. 23, 2230-2248.

Sarkar, B., Choi, H.L., Zhu, K., Mandal, A., Biswas, B., Suresh, A., 2016. Monitoring of soil biochemical quality parameters under greenhouse spinach cultivation through animal waste recycling. Chem. Ecol. 32, 407-418.

Schmidt, H.P., Kammann, C., Niggli, C., Evangelou, M.W.H., Mackie, K.A., Abiven, S., 2014. Biochar and biochar-compost as soil amendments to a vineyard soil: influences on plant growth, nutrient uptake, plant health and grape quality. Agric. Ecosyst. Environ. 191, 117-123.

Shaheen, S.M., Niazi, N.K., Hassan, N.E.E., Bibi, I., Wang, H., Tsang, D.C.W., Ok, Y.S., Bolan, N., Rinklebe, J., 2019. Wood-based biochar for the removal of potentially toxic elements in water and wastewater: a critical review. Int. Mater. Rev. 64 (4), 216-247.

Sun, X., Xu, Y., Zhang, Q., Li, X., Yan, Z., 2018. Combined effect of water inundation and heavy metals on the photosynthesis and physiology of *Spartina alterniflora.* Ecotox. Environ. Safe. 153, 248-258.

Tan, X., He, Y., Wang, Z., Li, C., Kong, L., Tian, H., Shen, W., Megharaj, M., He, W., 2018. Soil mineral alters the effect of Cd on the alkaline phosphatase activity. Ecotox. Environ. Safe. 161, 78-84.

Wang, Y., Xu, Y., Li, D., Tang, B., Man, S., Jia, Y., Xu, H., 2018. Vermicompost and biochar as bio-conditioners to immobilize heavy metal and improve soil fertility on cadmium contaminated soil under acid rain stress[J]. Sci. Total Environ. 621, 1057.

Wardle, D., Nilsson, M., Zackrisson, O., 2008. Fire-derived charcoal causes loss of forest humus.

564 Science 320(5876), 629-629.
565 Wei, J., Tu, C., Yuan, G., Bi, D., Xiao, L., Theng, B.K.G., Wang, H., Ok, Y.S., 2019. Carbon-coated
566 montmorillonite nanocomposite for the removal of chromium(VI) from aqueous solutions. J.
567 Hazard. Mater. 368, 541-549.
568 Wei, Y., Zheng, X., Shohag, M.J.I., Gu, M., 2017. Bioaccessibility and Human Exposure Assessment
569 of Cadmium and Arsenic in Pak choi Genotypes Grown in Co-Contaminated Soils. Int. J. Env.
570 Res. Pub. He. 14(9), 977.
571 Wu, P., Cui, P., Alves, M.E., Peijnenburg, W.J.G.M., Liu, C., Zhou, D., Wang, H., Ok, Y.S., Wang, Y.,
572 2019. Interactive effects of rice straw biochar and γ-Al2O3 on immobilization of Zn. J. Hazard.
573 Mater. 373, 250-257.
574 Wu, W., Li, J., Lan, T., Müller, K., Niazi, N.K., Chen, X., Xu, S., Zheng, L., Chu, Y., Li, J., Yuan, G.,
575 Wang, H., 2017. Unraveling sorption of lead in aqueous solutions by chemically modified biochar
576 derived from coconut fiber: A microscopic and spectroscopic investigation. Sci. Total Environ.
577 576, 766-774.
578 Yan, S., Ling, Q., Bao, Z., Chen, Z., Yan, S., Dong, Z., Zhang, B., Deng, B., 2009. Cadmium
579 accumulation in pak choi (*Brassica chinensisL.*) and estimated dietary intake in the suburb of
580 Hangzhou city, China. Food Addit. Contam. B. 2(1), 74-78.
581 Yang, X., Liu, J., Mcgrouther, K., Huang, H., Lu, K., Guo, X., He, L., Lin, X., Che, L., Ye, Z., 2016.
582 Effect of biochar on the extractability of heavy metals (Cd, Cu, Pb, and Zn) and enzyme activity in
583 soil. Environ. Sci. Pollut. Res. 23, 974-984.
584 Yang, X., Lu, K., McGrouther, K., Che, L., Hu, G., Wang, Q., Liu, X., Shen, L., Huang, H., Ye, Z.,
585 Wang, H., 2017. Bioavailability of Cd and Zn in soils treated with biochars derived from tobacco
586 stalk and dead pigs. J. Soils Sediments 17, 751-762.
587 Yang, X., Wan, Y., Zheng, Y., He, F., Yu, Z., Huang, J., Wang, H., Ok, Y.S., Jiang, Y., Gao, B., 2019.
588 Surface functional groups of carbon-based adsorbents and their roles in the removal of heavy
589 metals from aqueous solutions: A critical review. Chem. Eng. J. 366, 608-621.
590 Zhang, A., Bian, R., Pan, G., Cui, L., Hussain, Q., Li, L., Zheng, J., Zheng, J., Zhang, X., Han, X., Yu,
591 X., 2012. Effects of biochar amendment on soil quality, crop yield and greenhouse gas emission in
592 a Chinese rice paddy: A field study of 2 consecutive rice growing cycles. Field Crop Res. 127,
593 153-160.

Zhang, X., Wang, H., He, L., Lu, K., Sarmah, A., Li, J., Bolan, N.S., Pei, J., Huang, H., 2013. Using biochar for remediation of soils contaminated with heavy metals and organic pollutants. Environ. Sci. Pollut. Res. 20, 8472-8483.

Zhao, K., Fu, W., Qiu, Q., Ye, Z., Li, Y., Tunney, H., Dou, C., Zhou, K., Qian, X., 2019. Spatial patterns of potentially hazardous metals in paddy soils in a typical electrical waste dismantling area and their pollution characteristics. Geoderma 337, 453-462.

Zheng, H., Wang, Z., Deng, X., Herbert, S., Xing, B., 2013. Impacts of adding biocharon nitrogen retention and bioaccessibility in agricultural soil. Geoderma 206, 32-39.

Supporting Information: Appendix A

Animal carcass- and wood-derived biochars improved nutrients bioavailability, enzymes activity, and plant growth in metal-phthalic acid ester co-contaminated soils: A trial for reclamation and improvement of degraded soils

Hanbo Chen [a], Xing Yang [a,b], Hailong Wang [a,c*], Binoy Sarkar [d], Sabry M. Shaheen [b,e,f], Gerty Gielen [g], Nanthi Bolan [h], Jia Guo [i], Lei Che [j], Huili Sun [k], Jörg Rinklebe [b,l]

[a] *Biochar Engineering Technology Research Center of Guangdong Province, School of Environmental and Chemical Engineering, Foshan University, Foshan, Guangdong 528000, China*

[b] *University of Wuppertal, School of Architecture and Civil Engineering, Institute of Foundation Engineering, Water- and Waste Management, Laboratory of Soil- and Groundwater-Management, Pauluskirchstraße 7, 42285 Wuppertal, Germany*

[c] *Key Laboratory of Soil Contamination Bioremediation of Zhejiang Province, Zhejiang A&F University, Hangzhou, Zhejiang 311300, China*

[d] *Lancaster Environment Centre, Lancaster University, Lancaster, LA1 4YQ, UK*

[e] *King Abdulaziz University, Faculty of Meteorology, Environment, and Arid Land Agriculture, Department of Arid Land Agriculture, 21589 Jeddah, Saudi Arabia*

[f] *University of Kafrelsheikh, Faculty of Agriculture, Department of Soil and Water Sciences, 33 516 Kafr El-Sheikh, Egypt*

[g] *Scion, Private Bag 3020, Rotorua 3046, New Zealand*

[h] *Global Centre for Environmental Remediation, University of Newcastle, Newcastle, NSW, 2308, Australia*

[i] *Chengbang Eco-Environment Co. Ltd, Hangzhou, Zhejiang 310008, China*

[j] *School of Engineering, Huzhou University, Huzhou, Zhejiang 313000, China*

[k] *Key Laboratory of Marine Bio-resources Sustainable Utilization, South China Sea Institute of Oceanology, Chinese Academy of Sciences, Guangzhou, Guangdong 510301, China*

[l] *University of Sejong, Department of Environment, Energy and Geoinformatics, 98 Gunja-Dong, Guangjin-Gu, Seoul, South Korea*

[*]Corresponding author. E-mail: hailong.wang@fosu.edu.cn

1. Soil characterization and preparation

The soils (LOC and HOC) were characterized for their basic properties. The grain size distributions of the LOC and HOC soils were sand (55.0%, 46.5%), silts (26.1%, 34.9%) and clay (18.9%, 19.7%), respectively. Both soils were classified as clay loam ferrosols (Chen et al., 2019). Owing to the long-term intensive fertilization management, the HOC soil had a higher concentration of available K (150.37 mg kg^{-1}), N (174.86 mg kg^{-1}) and P (73.89 mg kg^{-1}), while the available K, N, P concentrations in LOC soil were 76.06 mg kg^{-1}, 23.34 mg kg^{-1} and 4.55 mg kg^{-1}, respectively.

Soil spiking with Cd and DEHP

A 100-mL methanol solution containing 5,400 mg of DEHP and a 100-mL deionized water containing 219.4 mg of $CdCl_2 \cdot 2.5H_2O$ were sprayed onto 3 kg of soil successively. The Cd/DEHP-spiked soils were mixed homogenously, air dried, and then gradually diluted with clean soil until the concentration of DEHP and Cd^{2+} was 50 mg·kg^{-1} and 1 mg·kg^{-1} in both soils, respectively. The concentration of Cd^{2+} was referred to the Level 3 standard of Environmental quality standard for soils GB 15618-1995, and concentration of DEHP was according to previous research (He et al., 2016). These concentration levels would markedly influence the normal plant growth but the plant could still develop normally.

2. Additional materials and methods

Soil properties determination

The soil pH value was measured by a 1/2.5 (w/v) soil suspension in deionized water with a pH electrode. The electrical conductivity (EC) value of soil was determined in a soil/water slurry at 1:5 (w/v) ratio using an EC meter. Soil particle composition was determined by hydrometer method. The soil organic carbon was determined by the potassium dichromate ($K_2Cr_2O_7$) and concentrated sulfuric acid (H_2SO_4) oxidation method (Lu, 1999). The cation exchange capacity (CEC) of soil was measured using 1 M ammonium acetate (pH 7) method (Lu, 1999).

Available and total Cd concentrations in soil

Potentially available Cd was extracted from 5 g air-dried soil with 25 mL diethylenetriaminepentaacetate acid (DTPA) solution and quantified using inductively coupled plasma optical emission spectroscopy (ICP-OES Optima 2000, PerkinElmer Co., USA) (Lu, 1999). The total Cd content of the soils was determined by digesting

662 the soils with HF-$HClO_4$-HNO_3 and analysing the digest by ICP-OES (Carignan and Tessier, 1988).

663

664 *DEHP extraction in soil*

665 Briefly, 2 g air-dried soil sample was extracted in the presence of 2 g anhydrous sodium sulfate (Na_2SO_4) with 2
666 times 20 mL acetone: petroleum ether 1:1 (v/v). The extraction method consisted of vortex oscillating for 1min,
667 ultrasound extraction for 10min at 25 °C, and centrifugation at 4000 rpm for 7 min. The two aliquots of
668 supernatants were vigorously shaken with 100 mL of 6% Na_2SO_4 solution and the organic layer separated. This
669 organic fraction was evaporated to dryness using N_2, dissolved in 1 mL n-hexane, filtered (MICRO PES 0.45
670 μm) and transferred into a GC vial. The GC vials were kept at -20 °C before GC analysis (Chen et al., 2019).
671 These samples were then analyzed using a Gas Chromatography (SHIMADZU, GC2010, Japan) equipped with
672 a Flame Ionization Detector (FID), HP-5 capillary column (30 m × 0.25 mm × 0.25 μm) and auto sampler under
673 the following operating conditions: inlet temperature 280ºC, FID temperature 300ºC, initial oven temperature
674 80ºC; and final oven temperature 290ºC. The injection volume was 1.0 μL and the typical retention time of
675 DEHP was 15.4 minutes. This DEHP detection method was able to detect concentrations ranging from 1.0 to
676 50.0 $mg \cdot L^{-1}$.

677

678 **3. Supporting results**

679

680 **Table S1** Selected properties of the high organic matter content (HOC) and low organic matter content (LOC)
681 soils

Soil	HOC	LOC
Sand (%)	46.5	55.0
Silt (%)	34.9	26.1
Clay (%)	19.7	18.9
pH	5.14	4.89
CEC[a] (cmol kg^{-1})	12.86	7.54
Electrical conductivity (dS m^{-1})	0.10	0.02
OC[b] (%)	3.08	0.75
Available-K (mg kg^{-1})	150.37	76.06
Available-N (mg kg^{-1})	174.86	23.34
Available-P (mg kg^{-1})	73.89	4.55
Total Cd (mg kg^{-1})	B.D.L. [c]	B.D.L. [c]
DEHP (mg kg^{-1})	Not detected	Not detected

682 [a] CEC: cation exchange capacity.
683 [b] OC: organic matter content.
684 [c] B.D.L.: Below detection limit (<0.01 mg L^{-1})

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Table S2 Selected properties of the pig biochar (PB) and wood biochar (WB)

Biochar	C (%)	H (%)	O (%)	N (%)	pH	Ash (%)	EC[a] (dS m^{-1})	CEC (cmol kg^{-1})	Available K (g kg^{-1})	Olsen P (g kg^{-1})	SA[b] (cmol kg^{-1})	SSA[c] (m^2 g^{-1})
PB	37.5	1.7	55.8	4.7	10.04	60.0	2.17	7.13	0.72	1.87	551	23.1
WB	81.3	2.2	15.7	0.5	9.47	6.6	0.22	1.11	0.23	0.12	144	124.8

[a] EC: electrical conductivity.

[b] SA: surface alkalinity.

[c] SSA: specific surface area.

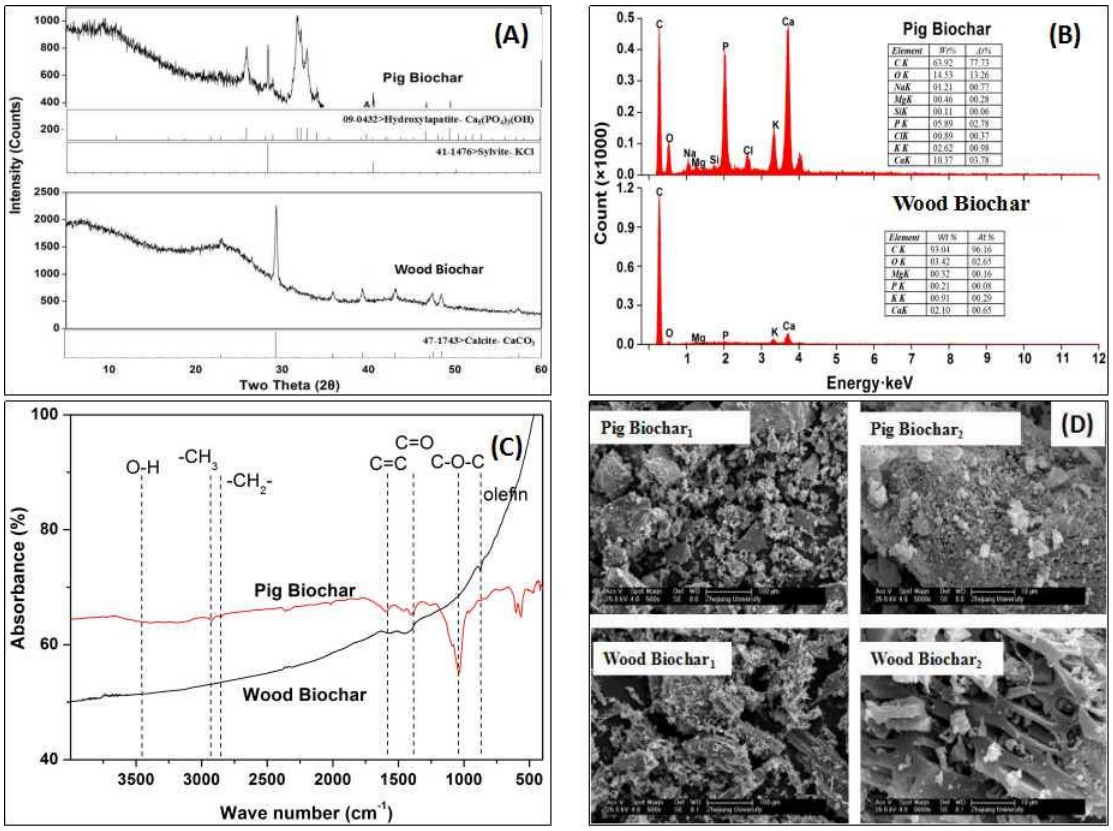

Fig. S1. X-ray diffraction (XRD) (A), energy dispersive X-ray spectrometry (EDS) (B), Fourier transform infrared (FTIR) spectrometry (C), and scanning electron microscope (SEM) images (D) of pig and wood biochars.

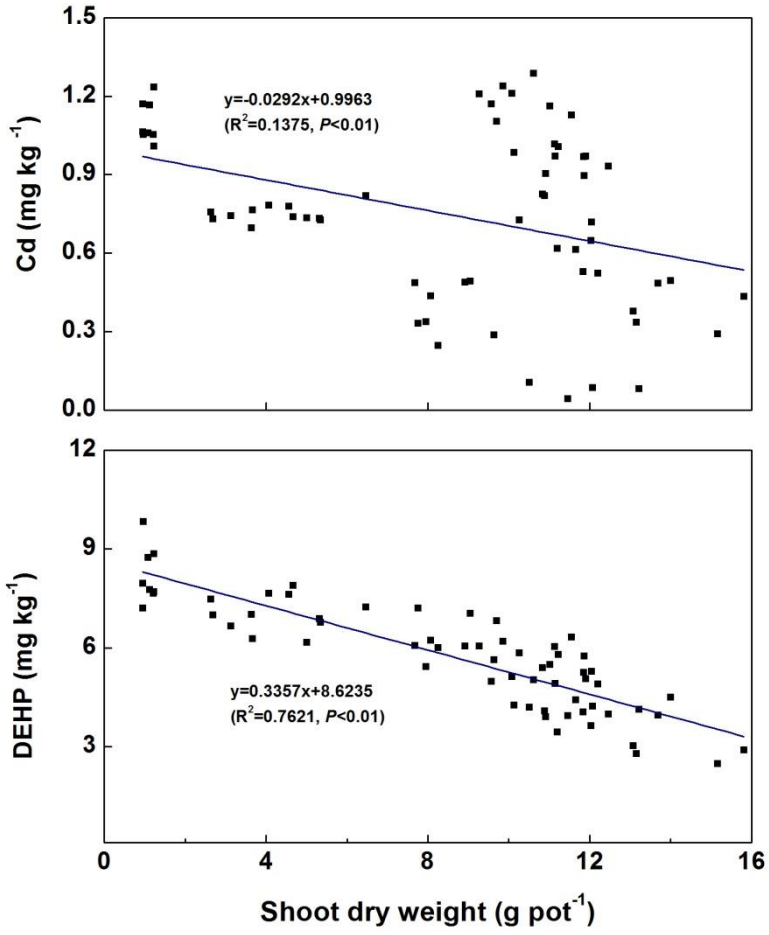

Fig. S2. Correlation between plant shoot dry weight and Cd and DEHP uptake by plants (n = 64).

References

Carignan, R., Tessier, A., 1988. The co-diagenesis of sulfur and iron in acid lake sediments of southwestern Québec. Geochim Cosmochim Ac 52, 1179-1188.

Chen, H., Yang, X., Gielen, G., Mandal, S., Xu, S., Guo, J., Shaheen, S.M., Rinklebe, J., Che, L., Wang, H., 2019. Effect of biochars on the bioaccessibility of cadmium and di-(2-ethylhexyl) phthalate to *Brassica chinensis* L. in contaminated soils. Sci. Total Environ. 678, 43-52.

He, L., Fan, S., Müller, K., Hu, G., Huang, H., Zhang, X., Lin, X., Che, L., Wang, H., 2016. Biochar reduces the bioaccessibility of di-(2-ethylhexyl) phthalate in soil. Chemosphere 142, 24-27.

Lu, R.K., 1999. Analytical Methods for Soil Agrochemistry. Chinese Agricultural Science and Technology Publishing House, Beijing (in Chinese).