

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

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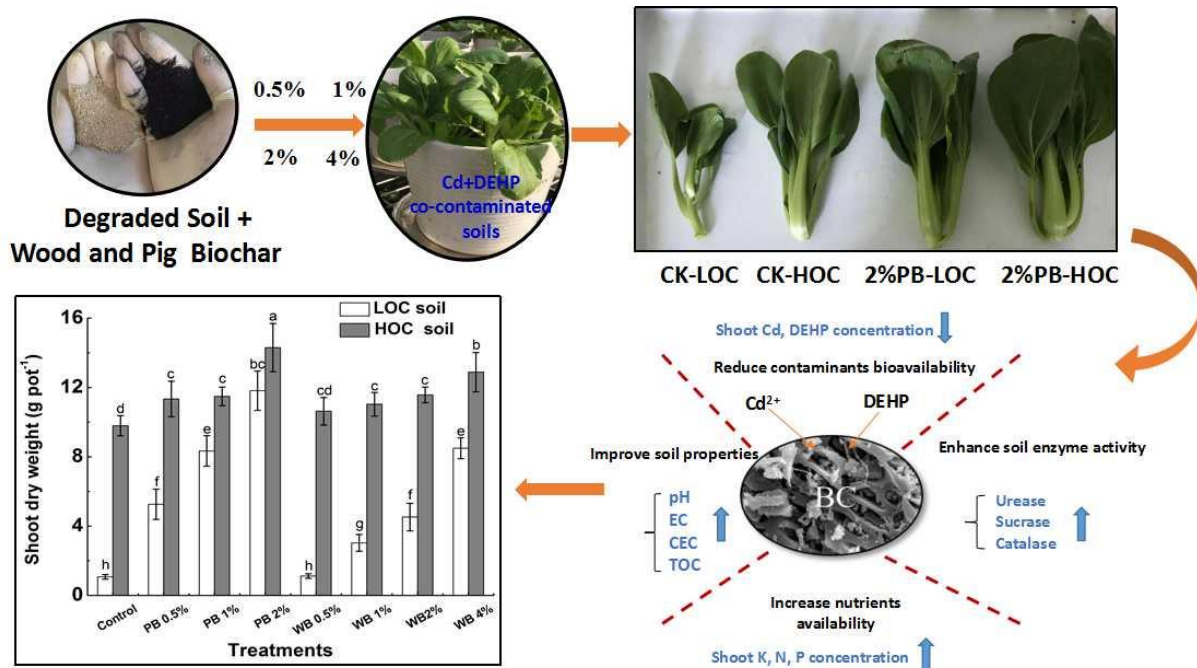
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29 **Graphical abstract**



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32 **Highlights**

- 33 • Biochar's effect on pak choi growth in Cd-DEHP co-contaminated soils was tested.
- 34 • 2% pig biochar addition increased the yield of pak choi.
- 35 • Pig biochar improved nutrient phytoavailabilities more than wood biochar.
- 36 • Tested biochars enhanced soil urease, sucrase and catalase activities.
- 37 • Biochars had prominent influence on pak choi growth in low organic carbon soil.

38

39 **Abstract**

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

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

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

61

62 **1. Introduction**

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

68 environmental and human health risks of these contaminants globally (Antoniadis et al., 2017; He et
69 al., 2015; He et al., 2018; Bandara et al., 2019). They can be taken up by plants, decreasing the yield
70 and quality of crops, and finally accumulated in human body through the food web, damaging the
71 functions of human organs including endocrine and reproductive systems (Qin et al., 2018; Chen et al.,
72 2019). Simultaneously, poor organic matter content of soils has been identified as a major reason for
73 loosing soil quality and crop yield worldwide (Pulido-Fernández et al., 2013). Therefore, reclamation
74 of degraded soils such as those co-contaminated with DEHP and heavy metal(loid)s, and soils with
75 low organic matter content is of great importance. Achieving such reclamation via suitable low-cost
76 amendments is an attractive option for soil restoration from both environmental quality and economic
77 points of view (Yang et al., 2016; Palansooriya et al., 2019, 2020).

78 Numerous studies describing biochar as a suitable material for remediating organic pollutants
79 (Zhang et al., 2013; Huang et al., 2018) and heavy metal(loid)s (Li et al., 2019a; Wu et al., 2017, 2019)
80 in water (Li et al., 2019b; Mao et al., 2019) and soils (Shaheen et al., 2019; Yang et al., 2019) have
81 been published. For instance, Abbas et al. (2017) found that the Cd concentration in wheat was
82 decreased after rice straw biochar amendment. They claimed that the probable reason could be the
83 reduction of Cd concentration in soil pore water for immediate crop uptake after biochar addition,
84 and/or biochar facilitated the combined effects of Cd bioavailability reduction and soil organic matter
85 improvement, as also suggested by Rizwan et al. (2016). In addition, biochar is of benefit to the
86 improvement of soil structure (i.e., aggregate formation) (Quan et al., 2020) and fertility (Li et al.,
87 2019c; Wei et al., 2019), and thereby promoting crop growth (Dong et al., 2015; Li et al., 2018;
88 Purakayastha et al., 2019).

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

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

96 In China, the area of vegetable cropping is second to grain production. Vegetables account for
97 approximately 28.5% of the total diet in China, and pak choi (*Brassica chinensis* L.) is a typical
98 widely-consumed leafy vegetable in daily life of the population (Yan et al., 2009). Wei et al. (2017)
99 noted that the consumption of pak choi as a staple vegetable made a significant contribution to the
100 estimated dietary intake of toxic metals such as Cd in Chinese population. As a consequence, it is of
101 importance to reduce contaminant accumulation in pak choi and improve the crop yield and quality. It
102 is well-accepted that soil enzymatic activity is sensitive to soil contaminants, accordingly considered
103 to be a crucial parameter of soil health (He et al., 2019). Soil enzymes also have a critical influence on
104 nutrient (e.g., K, N and P) cycling and subsequent uptake by plants (Sarkar et al., 2016; Nie et al.,
105 2018). Nutrient phytoavailability affects plant growth directly while contamination stress can inhibit
106 plant growth by posing toxic effects.

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

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

116

117 **2 Materials and methods**

118 **2.1 Soil and biochar collection, preparation, and characterization**

119 The studied soils were sampled from two near-by fields (0-20 cm of topsoil) located in the
120 southwest of Hangzhou City (30°24'N, 119°71'E), China. The first soil is rich in its total organic
121 carbon content (HOC: 3.08%) and was used as farmland to cultivate vegetables nearly for twenty
122 years. The second soil was left fallow for the same period, and thus was poor in its organic carbon
123 content (LOC: 0.75%). Both soils were air-dried, crushed, and sieved (3-mm mesh). In order to obtain
124 co-contaminated soils, the two soils were spiked with DEHP at 50 mg kg⁻¹ soil and Cd at 1.0 mg kg⁻¹.
125 The concentration of Cd²⁺ was referred to the Level 3 of the Environmental Quality Standards for
126 soils GB 15618-1995, and DEHP concentration was chosen according to a previous research (He et al.,
127 2016). The Cd/DEHP-spiked soils were mixed homogenously, air dried, and used for the pot
128 experiment.

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

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

138

139 **2.2 Pot trial**

140 The pot trial was carried out in a greenhouse located in Zhejiang A&F University, Zhejiang
141 Province, China, at temperature between 25 to 33°C. Each ceramic pot (20 cm diameter, 19 cm height)
142 was filled with either 3 kg of the Cd-DEHP contaminated LOC or HOC soil. Then the pig biochar and
143 wood biochar were applied to the Cd-DEHP contaminated soils in the ceramic pots at five doses (i.e.,
144 0, 0.5, 1, 2 and 4%, w/w) and mixed well. In total, eighteen treatments (including controls) were set in
145 this trial and every treatment repeated in four replicates. The LOC and HOC control soils did not
146 receive any dose of biochar.

147 All pots were fertilized with KH_2PO_4 and urea according to a basal dose of K_2O 0.2 $\text{g}\cdot\text{kg}^{-1}$, P_2O_5
148 0.32 $\text{g}\cdot\text{kg}^{-1}$, N 0.25 $\text{g}\cdot\text{kg}^{-1}$ recommended for pak choi (He et al., 2016). Treatments were arranged in a
149 complete randomized block design. The soil was maintained at 70% of the field water holding
150 capacity for an initial period of 30 days to equilibrate the spiked Cd and DEHP into the soil. After the
151 equilibration, ten pak choi seeds were sown at equal spacing in each ceramic pot on 10 July 2017.
152 After about fifteen days, five strongest seedlings were kept after thinning out the rest. Watering with
153 deionized water (2-3 times per week) was performed during the growth period to maintain the soil
154 moisture status at the field capacity. After maturity (50 days), the matured pak choi shoots were
155 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 105°C for 0.5 h and subsequently oven-dried at 65°C 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 (NaHCO₃)
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-HClO₄-HNO₃ (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 $\text{NH}_3\text{-N}$ 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**

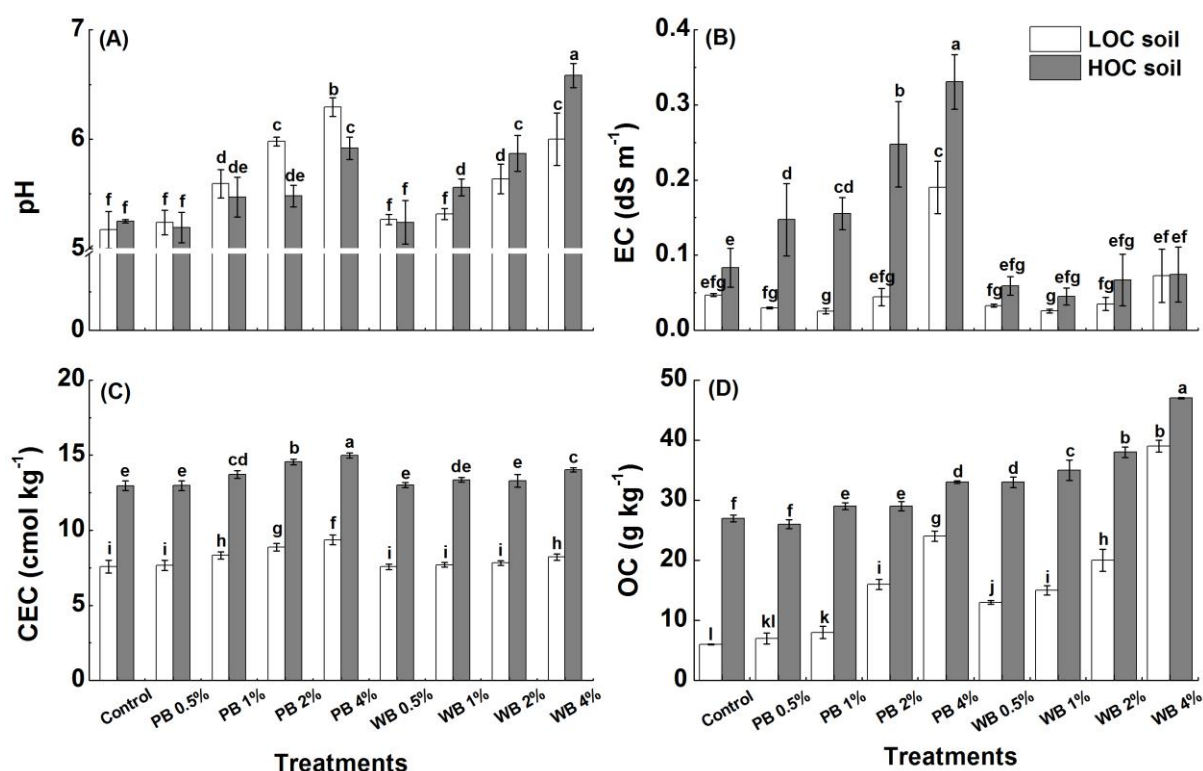
208 **3.1 Biochar-induced changes in soil pH, salinity, CEC, and organic carbon**

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

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

227



228 **Fig. 1.** Effect of biochar treatments on the pH (A), electrical conductivity (EC) (B), cation exchange
 229 capacity (CEC) (C), and organic carbon (OC) (D) in low organic carbon (LOC) soil and high organic
 230 carbon (HOC) soil. Control: untreated soil contaminated by Cd-DEHP; PB: pig biochar; WB: wood
 231 biochar. Error bars are standard deviation of the means (n=4). Different lower-case letters above the
 232 columns indicate significant difference between treatments ($P < 0.05$).
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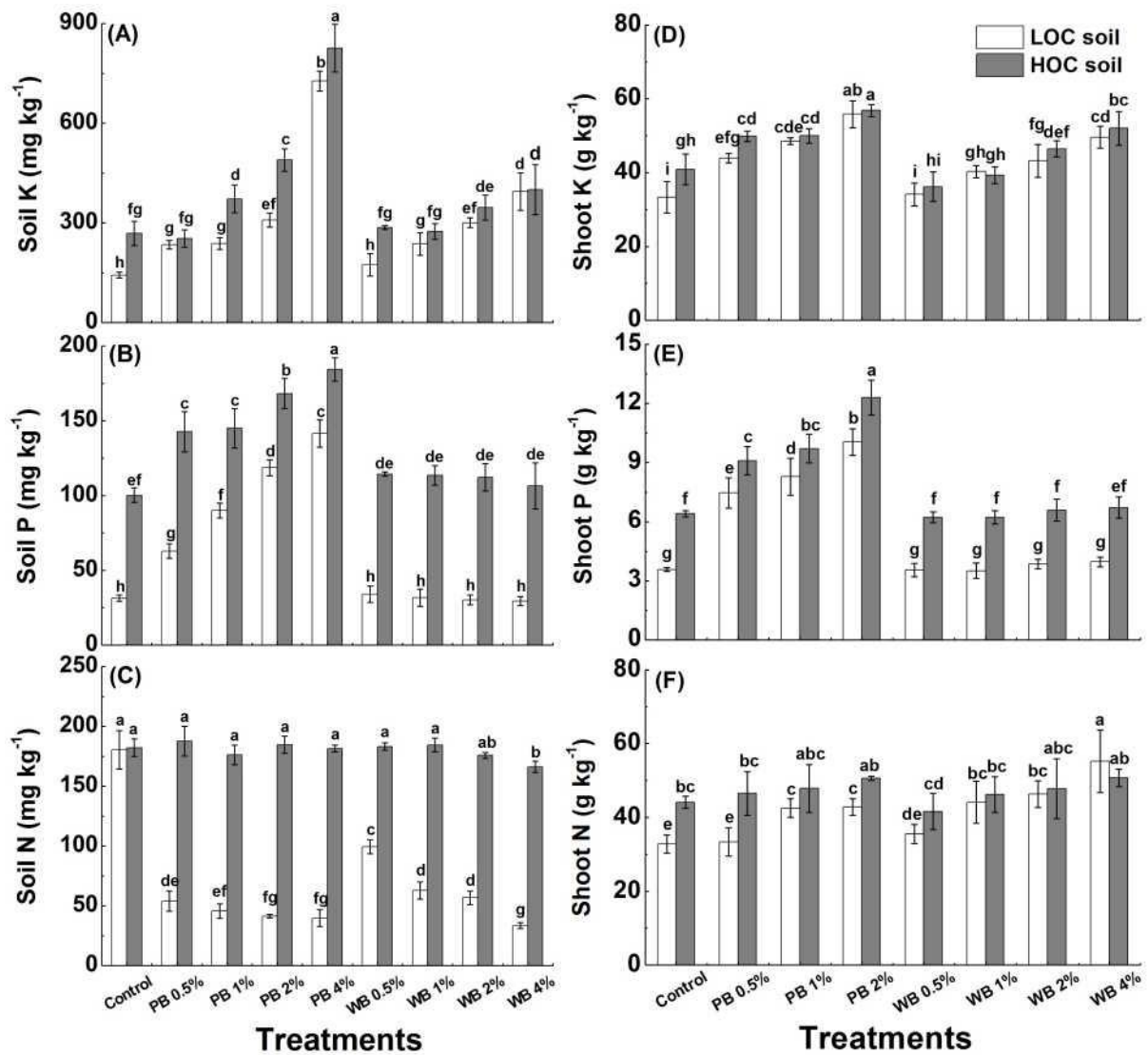
234

235 3.2 Impact of biochars on the bioavailability and uptake of N, P, and K

236 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.

246



247
 248 **Fig. 2.** Effect of biochar treatments on the available K (A), P (B) and N (C) in the low organic carbon
 249 (LOC) soil and high organic carbon (HOC) soil, and the uptake of K (D), P (E) and N (F) in plant
 250 shoots. Control: untreated soil contaminated by Cd-DEHP; PB: pig biochar; WB: wood biochar. Error
 251 bars are standard deviation of the means (n=4). Different lower-case letters above the columns
 252 indicate significant difference between treatments ($P < 0.05$).

253

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

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

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

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

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

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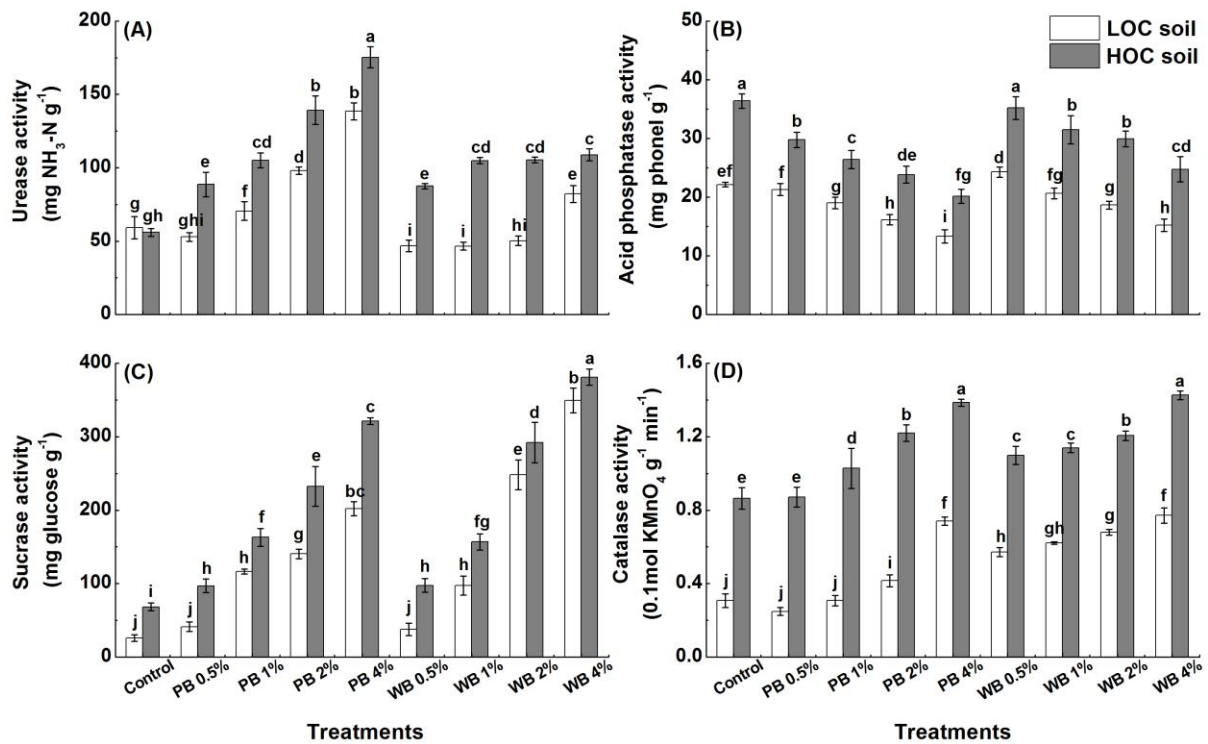
296 **3.3 Impact of biochars on enzyme activities**

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

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

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

318



319
 320 **Fig. 3.** Effect of biochar treatments on the activities of urease (A), acid phosphatase (B), sucrase (C)
 321 and catalase (D) in low organic carbon (LOC) soil and high organic carbon (HOC) soil. Control:
 322 untreated soil contaminated by Cd-DEHP; PB: pig biochar; WB: wood biochar. Error bars are
 323 standard deviation of the means (n=4). Different lower-case letters above the columns indicate
 324 significant difference between treatments ($P < 0.05$).

325
 326 Toxic metals, such as Cd ions, might deactivate the enzyme proteins, and thus inhibit soil
 327 enzymatic activities (Tan et al., 2018). Also, DEHP might affect the production of enzymes by causing
 328 dysfunction in the structure of cell membrane (Chen et al., 2019). Improving the activities of urease,
 329 sucrase, and catalase in the biochar treated soils as compared to the untreated soils indicated that both
 330 biochars mitigated the negative impact of Cd and DEHP on these enzyme activities in the
 331 contaminated soils. In our previous study (Chen et al., 2019), both the wood and pig biochars,
 332 particularly pig biochar, were able to reduce the bioavailability of Cd and DEHP in both soils.
 333 Therefore, we assume that the biochar-induced reduction of Cd and DEHP toxicity in
 334 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

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

362

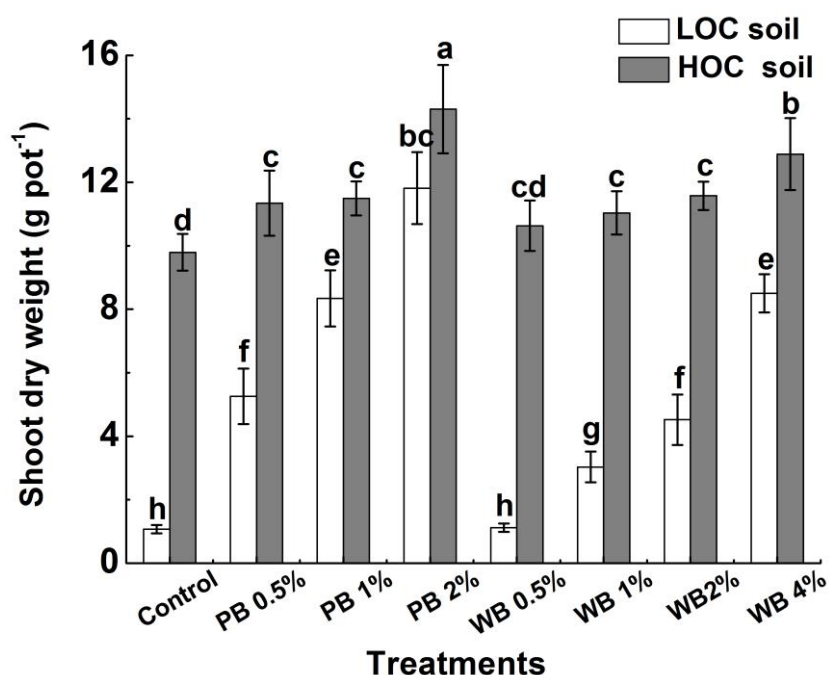
363 **3.4 Impact of biochars on plant growth**

364 Given that the seeds of pak choi in 4% pig biochar treatments did not germinate normally, we
365 eliminated the plant data from that treatment in the statistical analysis. The fact that pak choi did not
366 germinate well in the 4% pig biochar treatments in the pot trial suggested that high pig biochar dosage
367 (4%) inhibited plant growth, as observed also by Schmidt et al. (2014) and Khan et al. (2015).
368 Schmidt et al. (2014) reported that high biochar dosage might cause nutrient immobilization in soils,
369 particularly the dissolved organic carbon and mineral N, which consequently would restrict plant
370 growth. Another possible reason was that biochar application increased the available $\text{NH}_4^+\text{-N}$
371 concentration in the soil to a level which led to a stress condition for the plant (Khan et al., 2015).

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

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

383



384

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

389

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

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

400 In our previous study (Chen et al., 2019), we found that biochar application decreased Cd and
401 DEHP bioaccumulation in pak choi shoot, and the pig biochar application was more efficient in
402 comparison with wood biochar. The effect of biochars on the shoot dry weight was more prominent in
403 the LOC soil than in the HOC soil, which agrees with reports by Haefele et al. (2011) and Zhang et al.
404 (2012) who concluded that biochar produced from crop straw increased rice yield more significantly
405 in barren soils than fertile soils.

406

407 **4. Conclusions**

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

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

422

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427

428 **Appendix A. Supplementary data**

429 Supplementary data to this article can be found online.

430

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602

603 *Supporting Information: Appendix A*

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

607

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632 **1. Soil characterization and preparation**

633 The soils (LOC and HOC) were characterized for their basic properties. The grain size distributions of the LOC
634 and HOC soils were sand (55.0%, 46.5%), silts (26.1%, 34.9%) and clay (18.9%, 19.7%), respectively. Both
635 soils were classified as clay loam ferrosols (Chen et al., 2019). Owing to the long-term intensive fertilization
636 management, the HOC soil had a higher concentration of available K (150.37 mg kg⁻¹), N (174.86 mg kg⁻¹) and
637 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
638 4.55 mg kg⁻¹, respectively.

639

640 *Soil spiking with Cd and DEHP*

641 A 100-mL methanol solution containing 5,400 mg of DEHP and a 100-mL deionized water containing 219.4 mg
642 of CdCl₂·2.5H₂O were sprayed onto 3 kg of soil successively. The Cd/DEHP-spiked soils were mixed
643 homogenously, air dried, and then gradually diluted with clean soil until the concentration of DEHP and Cd²⁺
644 was 50 mg·kg⁻¹ and 1 mg·kg⁻¹ in both soils, respectively. The concentration of Cd²⁺ was referred to the Level 3
645 standard of Environmental quality standard for soils GB 15618-1995, and concentration of DEHP was according
646 to previous research (He et al., 2016). These concentration levels would markedly influence the normal plant
647 growth but the plant could still develop normally.

648

649 **2. Additional materials and methods**

650 *Soil properties determination*

651 The soil pH value was measured by a 1/2.5 (w/v) soil suspension in deionized water with a pH electrode. The
652 electrical conductivity (EC) value of soil was determined in a soil/water slurry at 1:5 (w/v) ratio using an EC
653 meter. Soil particle composition was determined by hydrometer method. The soil organic carbon was
654 determined by the potassium dichromate (K₂Cr₂O₇) and concentrated sulfuric acid (H₂SO₄) oxidation method
655 (Lu, 1999). The cation exchange capacity (CEC) of soil was measured using 1 M ammonium acetate (pH 7)
656 method (Lu, 1999).

657

658 *Available and total Cd concentrations in soil*

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

662 the soils with HF-HClO₄-HNO₃ 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 (Na₂SO₄) 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% Na₂SO₄ solution and the organic layer separated. This
669 organic fraction was evaporated to dryness using N₂, 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 280°C, FID temperature 300°C, initial oven temperature
674 80°C; and final oven temperature 290°C. 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·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)

685

686

687 **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

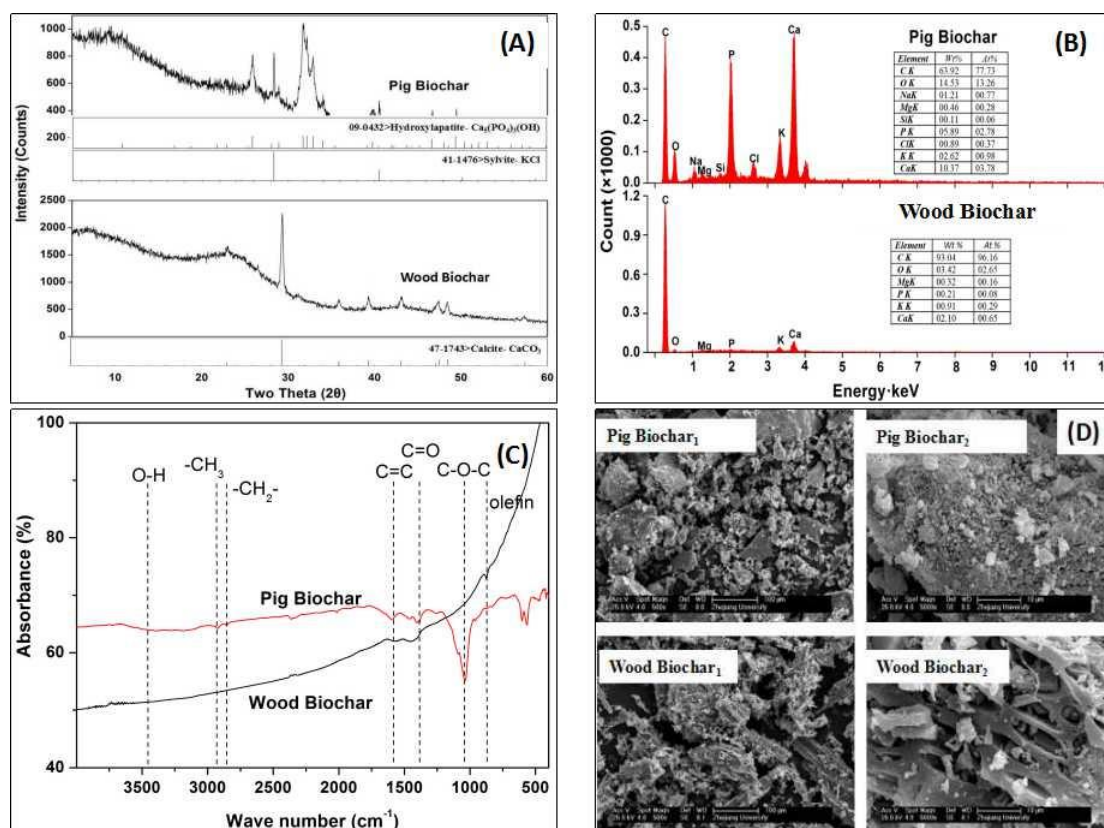
688 ^a EC: electrical conductivity.

689 ^b SA: surface alkalinity.

690 ^c SSA: specific surface area.

691

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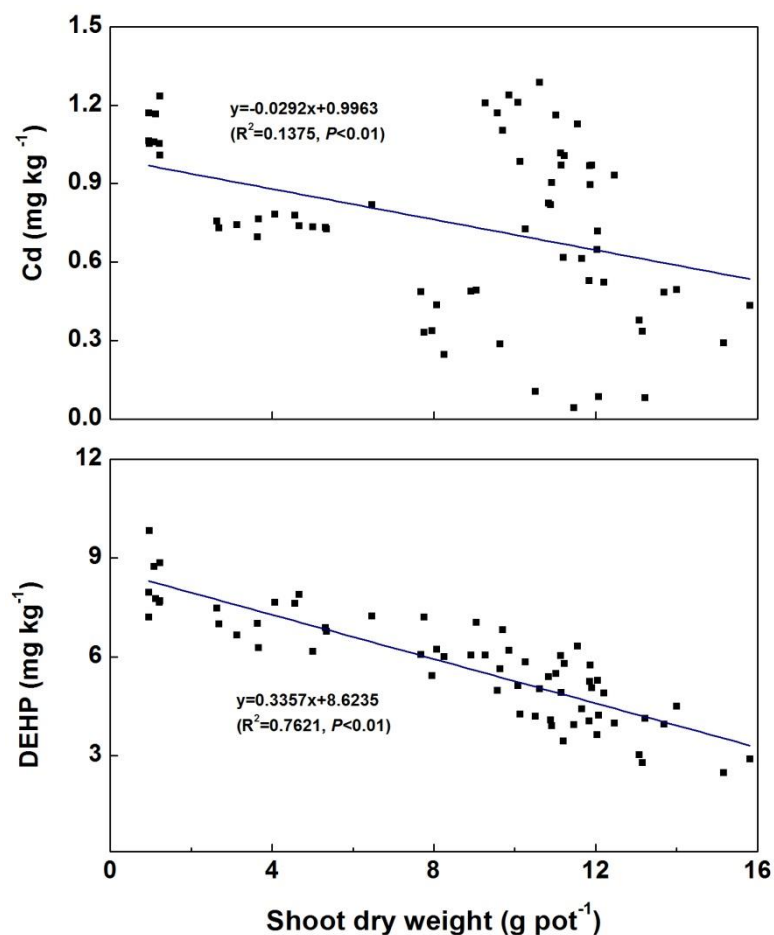


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694

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

698



699

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

701

702 **References**

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