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Fe-Mn impregnated biochar alleviates di-(2-ethylhexyl) phthalate stress in vegetative growth of wheat

YING LIU¹, ZHENG GUO SONG², LINSSEN BAI², XIPENG CHANG¹, YALEI XU¹,
MINLING GAO^{2*}

¹School of Environmental Science and Engineering, Tiangong University,
Xiqing District, Tianjin, P.R. China

²Department of Civil and Environmental Engineering, Shantou University,
Shantou, Guangdong Province, P.R. China

*Corresponding author: kyforever2013@163.com

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Abstract: In this study, we examined the effects of 0.5–2% iron and manganese oxide-modified biochar (FM) as remediation to control di-(2-ethylhexyl) phthalate (DEHP) in the soil and the response of wheat at different growth stages. The application of FM and original biochar (BC) significantly reduced DEHP concentrations in wheat roots and leaves and effectively immobilised DEHP in soils at different stages, and alleviated the oxidative damage of DEHP by significantly reducing O_2^- and H_2O_2 content and increasing the activities of antioxidant enzymes, including superoxide dismutase, catalase, and ascorbate peroxidase. Moreover, photosynthetic parameters (stomatal conductance, intercellular carbon dioxide concentration, photosynthetic rate, and transpiration rate) and fluorescence indicators (maximum photochemical efficiency, electron transport rate, and actual quantum yield) of the wheat growing in DEHP-spiked soils were also improved, which caused increases in the biomass of above-ground and underground at the seedling, booting, and ripening stages. Compared to BC, the FM amendment led to a greater improvement in crop biomass by reducing DEHP bioavailability. Therefore, FM has a good potential for the remediation of DEHP-polluted soils.

Keywords: plasticiser; Hapli-Udic Argosol; toxic effects; crop plants; *Triticum aestivum* L.

Di-(2-ethylhexyl) phthalate (DEHP) is one of the most common phthalates (PAEs), which is frequently detected in farmland because of the extensive application of plastic film and building materials in agricultural production practices. Because of its difficulty to form chemical bonds with polyvinyl chloride resin and strong hydrophobicity, DEHP easily flows into and adsorbs on soil particles and other environmental media, contributing to the pollution of plants and soil (Xie et al. 2020). Previous studies have revealed that DEHP inhibits photosynthesis, affects the biomass of crop plants, and stimulates the activities of superoxide dismutase (SOD) and

ascorbate peroxidase (APX) (Ma et al. 2013, Gao et al. 2019). Importantly, the consumption of food polluted with DEHP results in considerable ecotoxicity, mutagenicity, and teratogenic reproductive toxicity (Xie et al. 2020). As wheat is an essential crop that accounts for a high proportion of global food production, it is imperative to alleviate the biological toxicity of DEHP in wheat.

At present, amid growing awareness about the harmful effects of PAEs pollutants, remediation technologies have been developed based on physical, chemical, and biological principles to achieve the goal of reducing bioavailability (Zhang et al. 2020).

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Biochar is a carbonaceous porous material produced by the pyrolysis of biomass residues. Owing to its large specific surface area, biochar helps in remediation by trapping and deactivating pollutants, enhancing soil microbial biomass and activity, and increasing soil fertility, plant photosynthesis, and crop yields (Rizwan et al. 2019). However, the adsorption capacity of original BC is limited because of the absence of specific surface area and functional groups. Modifying and activating biochar to increase its adsorption capacity has been advocated as a novel approach to improve its ability to reduce the harmful effects of pollution. For example, the modification of biochar with chemical reagents such as acid or alkali can change its porous structure and increase the number of surface functional groups, thereby creating new binding sites for immobilising pollutants and thus reducing ecotoxicity (Liu et al. 2012). Meanwhile, the modification also leads to increased porosity and nutrient content which provides good pre-conditions for microbial activity (He et al. 2016).

Although biochar has been widely used as a soil amendment to reduce the bioavailability of heavy metals, there is limited information on its removal of organic pollutants during wheat growth. Our previous study showed that iron and manganese oxide-modified biochar (FM) and nano-manganese dioxide exhibited a relatively high absorption capability for DEHP and DBP in solution, respectively (Gao et al. 2017a, Guo 2020). Therefore, we speculated that FM could effectively decrease the uptake of DEHP by wheat plants grown in the DEHP-spiked soil. Our aims were to (1) examine the effects of FM on biomass, photosynthetic and biochemical parameters of wheat grown in DEHP-contaminated soils, and (2) investigate the impact of FM application on DEHP bioavailability.

MATERIAL AND METHODS

Soil collection and determination. The study used soils collected from agricultural fields (0–20 cm of topsoil) located in Liaoning province, China, which is classified as a Hapli-Udic Argosol (Cambisol, FAO-UNESCO 1988). The soils were air-dried, crushed, and sieved to a diameter of ≤ 2 mm. Soil organic carbon (SOC), available nitrogen (AN), available potassium (AK), Olsen phosphorus (OP), and pH were measured as described by Lu (2000). Soil is slightly acidic with a pH of 5.72, and SOC content is 10.77 g/kg. The content of AN, OP and AK is 60.12, 19.01 and 31.22 mg/kg, respectively.

Biochar production and modification. Original biochar (BC) from corn stalks was prepared for 2 h at 600 °C in a muffle furnace (MGS1300, Chengyi, Henan, China) under N_2 . BC (12.432 g) was immersed in $KMnO_4$ (0.24 mol/L, 40 mL) and $Fe(NO_3)_3$ (0.06 mol/L, 40 mL) and sonicated for 2 h, dried and pyrolysed for 0.5 h at 600 °C in an anaerobic environment. BC (FM) is slightly alkaline, with a pH of 8.90 (9.82). The content of C, N, H, and ash in BC (FM) is 76.05% (68.02%), 1.89% (1.76%), 2.99% (2.35%), and 16.01% (26.93%), respectively. The content of Fe in FM is 1.11%.

Pot trial. Eight litres of 150 mg/L DEHP methanol solution were gradually mixed with 120 kg of soil to achieve DEHP concentrations of 10 mg/kg and then air-dried for 5 days to completely volatilise the methanol and equilibrate the contaminated DEHP with the soil. The resultant concentration of DEHP was 8.06 mg/kg. Each pot was filled with 5 kg of DEHP-treated soil and mixed well with BC or FM at ratios of 0.5, 1, and 2%. DEHP-treated soils without BC or FM were used as the control. There were three replicates in all groups. Deionised water was added to maintain a 60% field capacity, and the pots were equilibrated for two weeks. Subsequently, a standard dose of 0.32 g/kg urea and 0.15 g/kg KH_2PO_4 was added to each pot as the base fertiliser. Wheat seeds (cv. Jinqiang 8) were sterilised with 30% H_2O_2 (v/v) for 30 min and washed with distilled water before sowing at the rate of 20 per pot. Water was replenished during the growth of the wheat at a rate of 100 mL per day before and 150 mL per day after flowering. Urea (2.15 g) and KH_2PO_4 (1.10 g) were applied to each treatment before booting. Wheat and soil samples were obtained at different stages and stored in foil pouches at -80 °C until analysis.

DEHP extraction and HPLC analysis. Soil samples (1.8 g) were sonically extracted with 20 mL CH_2Cl_2 for 10 min and centrifuged for 5 min at 10 000 g. Subsequently, the supernatant was concentrated in a rotary evaporator at 50 rpm in a 50 °C water bath. The residues were then dissolved in acetonitrile and filtered for high-performance liquid chromatography using an ultraviolet detector (Waters e2695 series; Waters, Inc., Milford, USA). The HPLC detector wavelength was 228 nm, and the column temperature was set at 25 °C. Acetonitrile and ultrapure water with 0.1% CH_3COOH were used as the mobile phase, and the flow rate was 0.8 mL/min. The injection volume was 20 μ L.

Wheat roots or leaves (0.5 g) were crushed into powder with a small amount of quartz sand and

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NaSO₄. The mortar was rinsed twice with a small amount of distilled water, and the mixture was sonicated with 20 mL CH₂Cl₂ for 20 min. Subsequently, the extractions were purified and rinsed three times with 5 mL CH₂Cl₂ using Al₂O₃ (bottom layer):NaSO₄ (top layer) = 3:1 by mass. Subsequently, the eluates were combined, concentrated, and DEHP contents were determined using the methods described above.

Measurement of photosynthetic parameters and chlorophyll fluorescence. Prior to sample collection at the seedling and booting periods, the net photosynthetic rate (P_n), transpiration rate (T_r), stomatal conductance (g_s), and intercellular CO₂ concentration (C_i) of a completely expanded functional second leaf (counted from the top) were analysed between 9.00 and 12.00 a.m. by a portable photosynthesis system (Li-6400XT; Li-COR, Lincoln, USA). The parameters of the analyser were set to the photosynthetic photon flux density of 1 000 μmol/m²/s, vapour pressure deficit of 1 kPa, and an airflow rate of 500 mmol/s. The temperature was 25 °C, carbon dioxide content was 458 μmol/mol, and light intensity were 1 000 μmol/m²/s.

Wheat plants were kept in the complete dark for half an hour before sampling; then, chlorophyll fluorescence was determined using the PAM-2000 photosynthesis determination system (Walz GmbH, Effeltrich, Germany). Pulses of saturated light (25 kHz, photosynthetic photon flux = 8 000 μmol/m²/s) of 3-μs duration were employed to measure the maximum (F_m) and minimum (F_0) fluorescence; the maximum photochemical efficiency (F_v/F_m) of the PS II was computed as $F_v/F_m = (F_m - F_0)/F_m$. The actual quantum yield (ϕ_{PSII}) and photosynthetic electron transfer rate (ETR, μmol CO₂/m²/s) of PS II were also determined.

Biomass. Three wheat plants in each treatment were collected, excluding soil on roots; the samples were washed with distilled water and dried with filter paper. Subsequently, the wheat tissues were fixed for 0.5 h at 105 °C and dried to a constant weight at 80 °C.

Assays of ROS content and antioxidant enzyme activity. Fresh wheat leaves and roots (0.25 g) were completely ground under the protection of liquid nitrogen and transferred to a centrifuge tube with 3 mL of 0.9% saline. After extraction, the samples were centrifuged for 20 min at 8 000 g and 4 °C. The supernatant was adopted as the raw enzyme extraction, and the contents of H₂O₂ and O₂⁻, SOD, CAT, and APX in extractions were analysed using

a microplate reader (Infinite 200 PRO, Tecan, Mannedorf, Switzerland) based on the instructions from the manufacturer (Nanjing Jiancheng Bioengineering Institute, Nanjing, China).

Statistical analysis. Differences among groups were calculated using an ANOVA performed by SPSS 25.0 (SPSS Inc., Chicago, USA) and recognised as statistically significant at $P < 0.05$. Data are shown as mean ± standard deviation.

RESULTS AND DISCUSSION

Effects of FM addition on the bioavailability of DEHP in soils and wheat. DEHP, a typical class of PAEs, accumulates in roots through the root cortex and is also transported to the stem and leaves (Gao et al. 2017b). This stresses all aspects of the wheat plant, including root morphology, oxidative stress, accumulation of biomass, and capacity for photosynthesis. Biochar, in concert with other charcoal-based adsorbents, can reduce the contents of organic pollutants that can bioaccumulate in crop plants. In this study, we found that, compared to plants grown in the control soils, BC and FM amendments significantly reduced the uptake of DEHP by wheat leaves and roots (except in the ripening stage) at different stages of growth (Figure 1A, $P < 0.05$). Compared to the control, BC application decreased DEHP content in leaves (and roots) by 6.18–20.9% (20.3–49.3%), 24.4–49.7% (18.4–36.7%), and 4.22–17.7% (10.1–43.2%) at the seedling, booting and ripening stages, respectively, while FM application declined DEHP content in leaves (and roots) by 16.3–30.6% (34.4–70.1%), 30.0–60.8% (30.2–60.5%), and 16.3–30.6% (17.4–45.0%) at the seedling, booting and ripening stages, respectively, compared to the control. The results indicated that BC and FM supplements effectively improved the DEHP accumulation in wheat tissues because of their relatively high surface area and alkaline nature (Gao et al. 2021). This result was similar to the previous study, which indicated that the addition of BC to soil enhanced the adsorption of DEHP and decreased the desorption and flow activity in the soil, thus reducing the degradation of DEHP in soil and its uptake by crop plants (Zhang et al. 2014).

In the current study, there was a higher DEHP concentration in the soils with increasing FM and BC doses compared to those in the control soils (Figure 1B). It is possible that the formation of H-bonds between the O atom of the -COOR in PAEs and the functional groups of BC and FM may reduce the

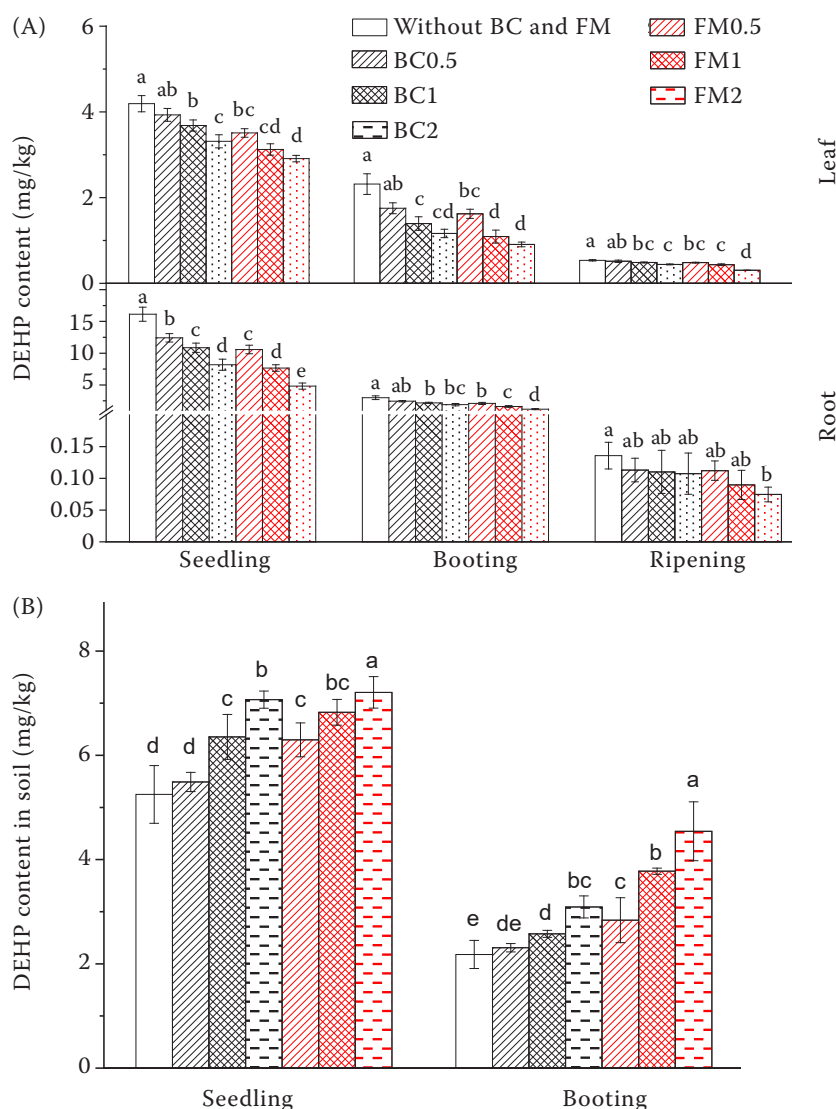
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Figure 1. Effects of biochar (BC) and Fe-Mn impregnated biochar (FM) on the di-(2-ethylhexyl) phthalate (DEHP) content in (A) wheat tissues and (B) soils at a different stage. The numbers 0.5, 1 and 2 represent the 0.5, 1 and 2% ratio of BC or FM. Different lowercase letters at the same stage indicate significant differences among groups ($P < 0.05$)

mobility and degradation of PAEs in soils (Gao et al. 2021). Similarly, He et al. (2016) observed that bamboo sawdust and rice straw BC prolonged the degradation of DEHP in soil and successfully prevented its uptake by *B. chinensis* L. Importantly, the application of FM produced a better reduction of DEHP bioavailability than did the BC treatments. FM may have higher porosity and specific surface area than ordinary BC, thereby achieving excellent physical adsorption of pollutants (Jiang et al. 2020). The introduction of FeO_x and MnO_x produced new functional groups on the carbon material surface, which may help to achieve more stable or irreversible chemisorption (Zhang et al. 2016). In addition, FeO_x on FM might form iron patches on the root surface, which may explain the inhibition of toxic substances from entering the cortex and preventing plant growth. Additionally, we found that the con-

centrations of DEHP in soils without and with BC and FM significantly decreased with the growth of the wheat. This was attributed to microbial degradation and hydrolysis of DEHP during the experiment.

Effects of addition of FM on photosynthesis and chlorophyll fluorescence. Photosynthesis is the drive of the synthesis and accumulation of organic matter and thus determines the growth and development of plants (Qiu et al. 2013). Therefore, we observed that the effects of the application of FM and BC on photosynthetic indicators in wheat leaves under DEHP stress. The results demonstrated that soil amendment with 1–2% BC (FM) significantly increased P_n of wheat leaves under DEHP stress by 13.2–16.9% (24.7–34.3%) and 11.4–12.7% (16.1–19.7%) at the seedling and booting stages, respectively ($P < 0.05$, Table 1). The enhancement in P_n could be explained by increased g_s and T_r following BC and FM applica-

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Table 1. Effect of biochar (BC) and Fe-Mn modified biochar (FM) on photosynthesis of wheat grown in di-(2-ethylhexyl) phthalate-contaminated soil

Stage	Treatment	Photosynthetic parameter				Chlorophyll fluorescence		
		P _n	g _s	T _r	C _i	F _m	Φ _{PSII}	ETR
Seedling	control	16.6 ± 0.71 ^d	0.33 ± 0.01 ^d	5.85 ± 0.22 ^c	204.49 ± 3.60 ^c	0.75 ± 0.01 ^d	0.69 ± 0.02 ^d	38.18 ± 1.35 ^d
	BC0.5	17.8 ± 1.37 ^{cd}	0.37 ± 0.02 ^c	6.30 ± 0.30 ^{bc}	218.73 ± 6.12 ^{bc}	0.77 ± 0.01 ^{cd}	0.72 ± 0.04 ^{cd}	41.16 ± 1.50 ^{cd}
	BC1	18.8 ± 0.56 ^{bc}	0.41 ± 0.02 ^{bc}	6.45 ± 0.40 ^{ab}	231.24 ± 17.55 ^b	0.79 ± 0.01 ^c	0.74 ± 0.01 ^{bc}	42.99 ± 2.58 ^c
	BC2	19.4 ± 1.34 ^{bc}	0.34 ± 0.01 ^b	6.57 ± 0.21 ^{ab}	237.23 ± 14.75 ^b	0.80 ± 0.02 ^{bc}	0.74 ± 0.01 ^{bc}	43.83 ± 1.95 ^{bc}
	FM0.5	18.4 ± 1.41 ^{cd}	0.38 ± 0.01 ^c	6.38 ± 0.29 ^{bc}	222.37 ± 10.20 ^{bc}	0.78 ± 0.02 ^{cd}	0.73 ± 0.02 ^{bc}	42.56 ± 2.54 ^c
	FM1	20.7 ± 0.92 ^{ab}	0.46 ± 0.02 ^a	6.77 ± 0.36 ^{ab}	263.12 ± 12.78 ^a	0.83 ± 0.02 ^{ab}	0.76 ± 0.02 ^{ab}	47.33 ± 2.12 ^{ab}
	FM2	22.3 ± 1.27 ^a	0.48 ± 0.02 ^a	7.00 ± 0.32 ^a	278.01 ± 11.33 ^a	0.85 ± 0.04 ^a	0.78 ± 0.04 ^a	49.78 ± 3.68 ^a
Booting	control	22.9 ± 1.01 ^c	0.63 ± 0.01 ^d	11.02 ± 0.24 ^c	324.59 ± 13.11 ^c	0.80 ± 0.02 ^d	0.72 ± 0.03 ^b	41.25 ± 1.16 ^d
	BC0.5	24.5 ± 1.18 ^{bc}	0.66 ± 0.026 ^{cd}	11.59 ± 0.27 ^{bc}	244.90 ± 7.56 ^{bc}	0.82 ± 0.01 ^c	0.74 ± 0.02 ^{ab}	43.41 ± 0.70 ^{cd}
	BC1	25.5 ± 0.96 ^{ab}	0.71 ± 0.035 ^{bc}	11.68 ± 0.21 ^b	351.21 ± 13.46 ^b	0.83 ± 0.01 ^c	0.75 ± 0.04 ^{ab}	45.32 ± 1.36 ^{bc}
	BC2	25.8 ± 2.07 ^{ab}	0.71 ± 0.040 ^{bc}	11.75 ± 0.47 ^b	354.58 ± 12.15 ^b	0.83 ± 0.01 ^{bc}	0.76 ± 0.03 ^{ab}	40.00 ± 0.87 ^{bc}
	FM0.5	24.9 ± 0.84 ^{bc}	0.69 ± 0.078 ^{cd}	11.61 ± 0.39 ^{bc}	347.73 ± 8.49 ^b	0.83 ± 0.01 ^c	0.75 ± 0.03 ^{ab}	46.15 ± 1.47 ^b
	FM1	26.6 ± 0.9 ^{ab}	0.77 ± 0.021 ^{ab}	12.48 ± 0.32 ^a	376.51 ± 16.23 ^a	0.85 ± 0.02 ^{ab}	0.76 ± 0.03 ^{ab}	44.23 ± 3.77 ^{cd}
	FM2	27.4 ± 1.05 ^a	0.81 ± 0.040 ^a	12.86 ± 0.37 ^a	388.97 ± 11.35 ^a	0.86 ± 0.02 ^a	0.80 ± 0.02 ^a	48.05 ± 1.72 ^a

The numbers 0.5, 1 and 2 represent the 0.5, 1 and 2% ratio of BC or FM. Different lowercase letters at the same stage indicate significant differences among groups ($P < 0.05$). P_n – net photosynthetic rate (μmol CO₂/m²/s); g_s – stomatal conductance (μmol H₂O/m²/s); T_r – transpiration rate (mmol H₂O/m²/s); C_i – intercellular carbon dioxide concentration (μmol CO₂/m²/s); F_m – maximum photochemical efficiency; Φ_{PSII} – actual quantum yield; ETR – electron transport rate (μmol CO₂/m²/s)

tion. The increasing g_s and T_r could be attributed to (1) a reduction in oxidative stress and DEHP uptake by wheat plants after BC and FM addition; (2) the BC and FM induced an increase in soil alkalinity and water holding capacity, which have been demonstrated to strongly correlate with the accumulation of P_n in C₃ plants (Speratti et al. 2018, He et al. 2020). The C_i is a major stomatal indicator that is used to determine whether the photosynthetic rate is changing because of stomatal factors. In this study, the change in C_i of wheat leaves treated with BC and FM was similar to that of P_n and g_s. Therefore, we considered that stomatal limitation might be responsible for the increase in P_n. These promotion effects are consistent with the previous findings (Rajendran et al. 2019, Irshad et al. 2020), who observed that the positive impact of BC on plant photosynthesis largely depends on alleviating soil constraints and reducing plant uptake of pollutants.

Chlorophyll fluorescence is a fundamental indicator that represents the absorption and utilisation of light energy in PSII (Goltsev et al. 2016). In this study, BC or FM application at 1% and 2% also showed a significant effect on F_v/F_m, Φ_{PSII} and ETR at the seedling and booting stages, but there were no significant changes at 0.5% BC and FM treatments (Table 1). It may be due to the alleviation of soil physicochemical characteristics in BC and FM treatments that finally raised a higher nitrogen accumulation in leaves and consequently increased F_v/F_m and Φ_{PSII} (Lin et al. 2013). Additionally, BC and FM addition may improve photosynthetic function in leaves by increasing chlorophyll content and subsequently inducing the synthesis of various enzymes and electron transporter during photosynthetic carbon assimilation (Hou et al. 2021). Further study is needed for the detailed mechanism.

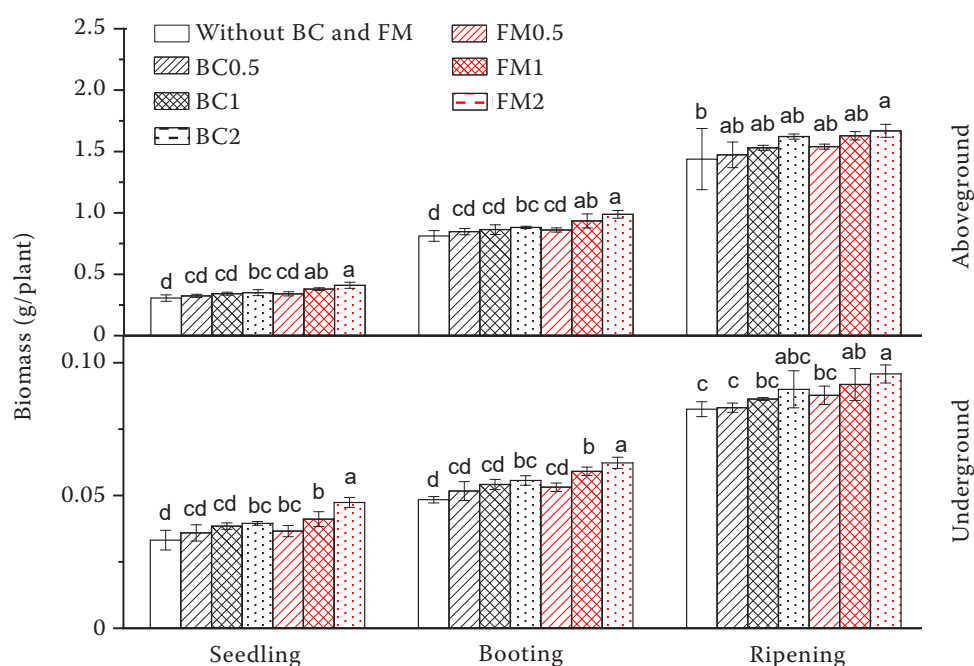


Figure 2. Effects on biochar (BC) and Fe-Mn impregnated biochar (FM) addition on the biomass (dry weight) of wheat under di-(2-ethylhexyl) phthalate (DEHP) stress at different stages. The numbers 0.5, 1 and 2 represent the 0.5, 1 and 2% ratio of BC or FM. Different lowercase letters at the same stage indicate significant differences among groups ($P < 0.05$)

Importantly, the FM treatment led to a greater improvement for photosynthesis and chlorophyll fluorescence indicators than BC treatments, particularly FM at 2%. Similarly, Irshad et al. (2020) found that the addition of 1.5% goethite-modified BC could increase the photosynthetic activity of rice planted in chromium arsenic-contaminated soil by 73.3% compared to 32% enhanced photosynthetic activity using 1.5% BC. We considered that Fe and Mn carried by FM might also have a non-negligible stimulatory effect. Liu et al. (2017) found that the application of iron materials enhanced the photosynthetic activity and gas exchange properties of rice plants grown in cadmium-contaminated soils. Mn catalysed the photo-induced decomposition of water molecules in photosystem II (PSII) and RuBP carboxylase reactions, both of which are important for photosynthesis (Marschner 1986). Moreover, FM had a greater specific surface area than BC; therefore, it reduced the uptake of DEHP by the wheat.

Effects of FM application on biomass. It is well known that DEHP interventions are significantly negatively correlated with the accumulation of plant biomass, and they may also be correlated with plant longevity (Ma et al. 2018). Encouragingly, we found that the application of FM and BC to

DEHP-contaminated soil gradually ameliorated the effects of DEHP on the biomass of wheat above-ground and underground at the seedling, booting, and ripening stages (Figure 2). BC application at 2% significantly increased the above-ground and underground biomass at different periods ($P < 0.05$) compared to that of the control. Noticeably, 1% (2%) FM application significantly increased the above-ground biomass by 23.9% (33.9%), 15.1% (21.6%), and 13.2% (16.0%) at seedling, booting and ripening stages ($P < 0.05$), respectively, and the root biomass significantly increased by 23.8% (42.8%), 22.0% (28.6%), and 11.3% (16.1%), respectively, compared to the control. This may be because the addition of BC and FM directly improved soil cation exchange capacity, pH, porosity, bulk density, and water content and effectively diminished the loss of P and N in the BC-soil system, which in turn improved the nutritional conditions for plants (Ahmed et al. 2016, Gao et al. 2021). Chen et al. (2020) found that the application of BC elevated the biomass of pak choi (*Brassica chinensis* L.) under DEHP and Cd stress; they attributed this effect to improved effectiveness of soil nutrients, increased pH, and improved fixation of contaminants in soils by sorption. Moreover, FM and BC reduced the bioavailability of DEHP in

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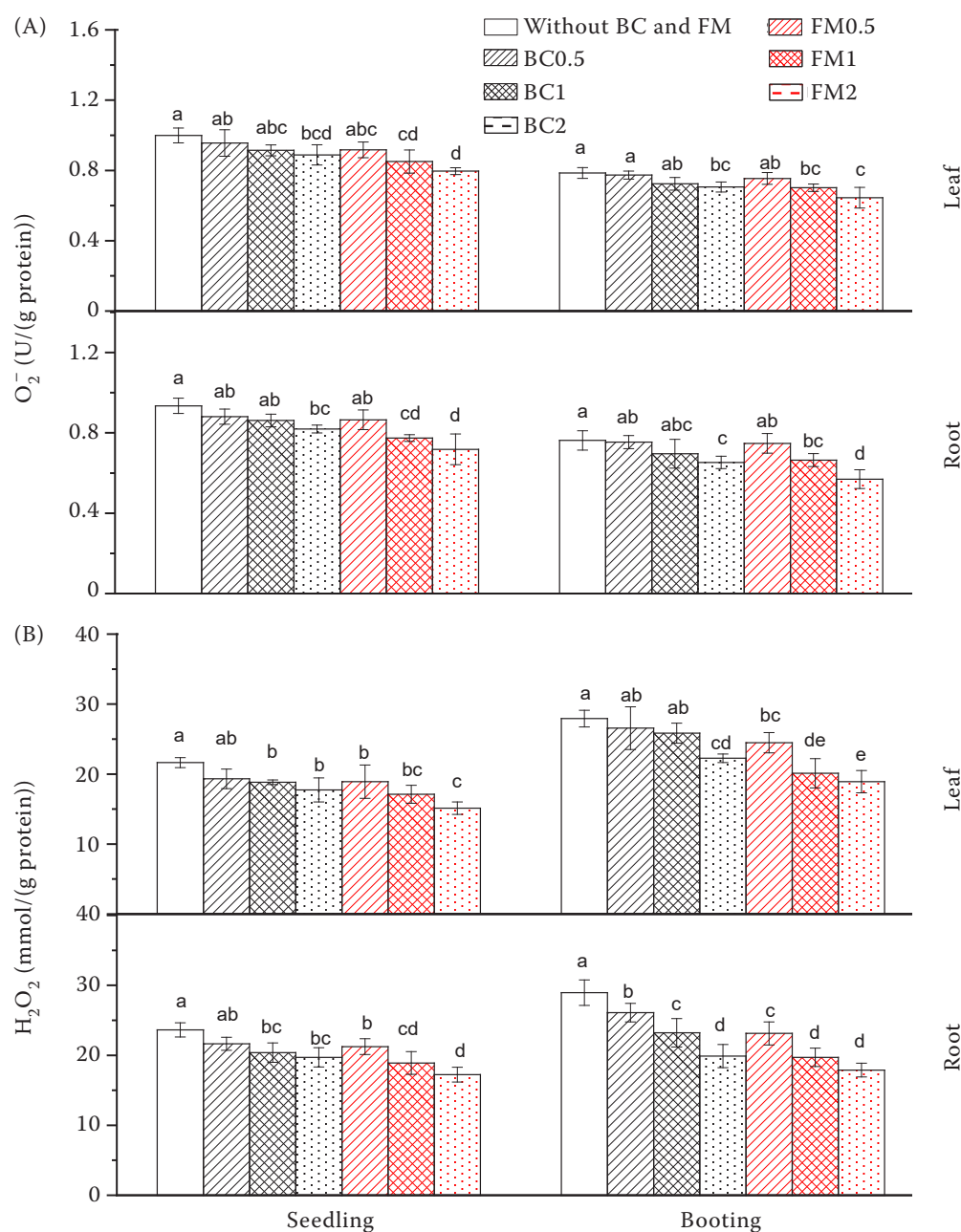


Figure 3. Effects of biochar (BC) and Fe-Mn impregnated biochar (FM) on (A) O_2^- and (B) H_2O_2 in wheat tissues under di-(2-ethylhexyl) phthalate (DEHP) stress. The numbers 0.5, 1 and 2 represent the 0.5, 1 and 2% ratio of BC or FM. Different lowercase letters at the same stage indicate significant differences among groups ($P < 0.05$)

soil, which caused a lower accumulation of DEHP in wheat plants. This may be a major reason for the increase in root and shoot biomass. Jan et al. (2020) reported that the effect of corn waste biochar as a soil conditioner was to promote the immobilisation of Cd, reduce the bioavailability of Cd in soil, and promote the growth of wheat. Additionally, photosynthesis is a vital physiological process in plant productivity, contributing 90–95% of the crop yield.

A previous study has found that BC has a positive effect on photosynthetic rate and chlorophyll, which significantly increases crop yield (Zhu et al. 2019). Finally, BC and FM amendments could stimulate the production of growth-promoting hormones that might contribute to the development of the plant (Viger et al. 2015). In the current study, FM application induced a significant enhancement in wheat biomass than that of BC. It could be at-

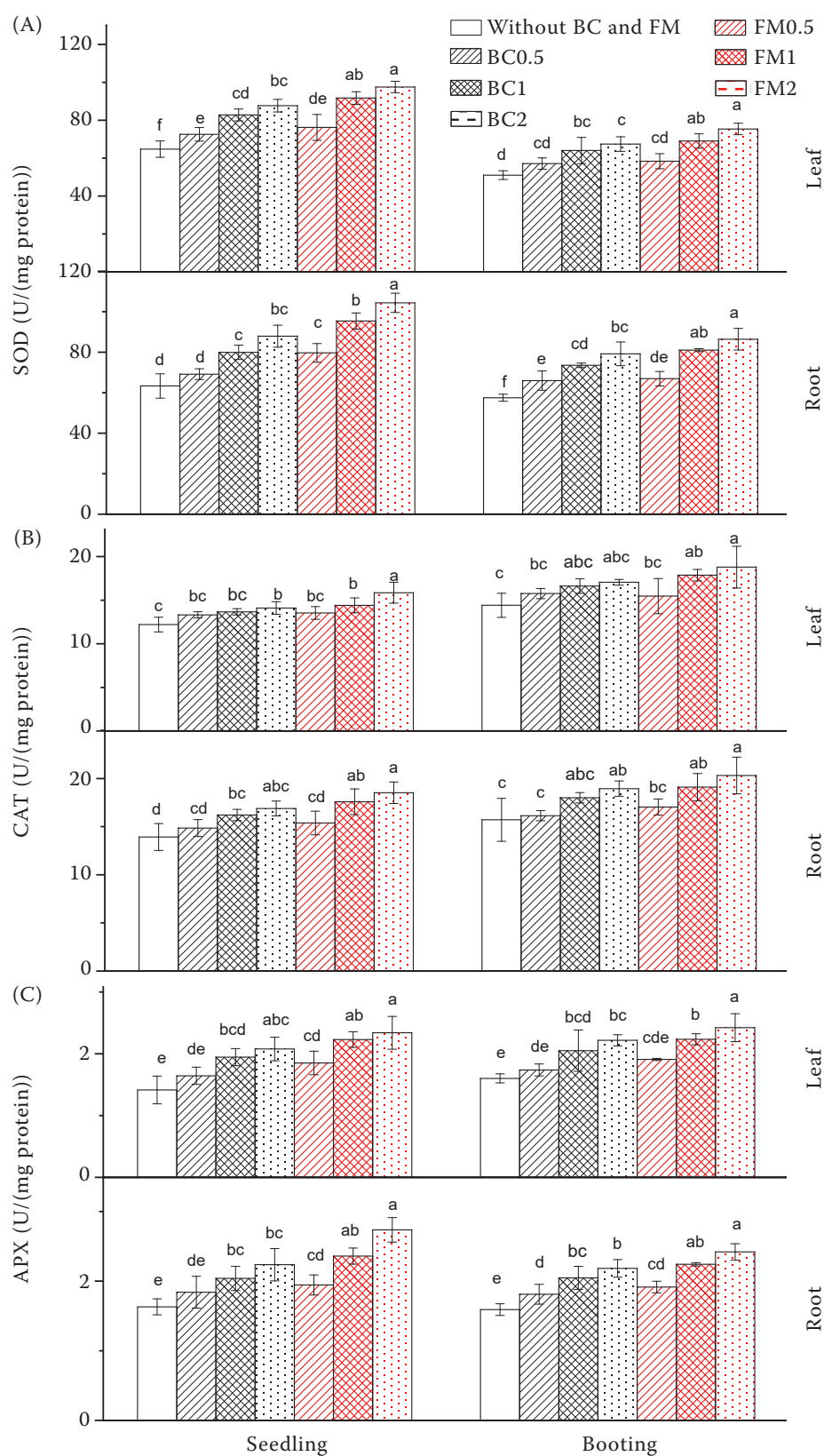


Figure 4. Effects of biochar (BC) and Fe-Mn impregnated biochar (FM) on enzyme activity of wheat grown in di-(2-ethylhexyl) phthalate (DEHP) contaminated soils at different stages. The numbers 0.5, 1 and 2 represent the 0.5, 1 and 2% ratio of BC or FM. Different lowercase letters at the same stage indicate significant differences among groups ($P < 0.05$). SOD – superoxide dismutase; CAT – catalase; APX – ascorbate peroxidase

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tributed to lower DEHP accumulation and higher photosynthesis in FM treatments. A similar result was reported by Irshad et al. (2020), who observed that goethite-modified BC significantly increased the biomass of rice plants under Cd and As co-contaminated soil than that of the original BC.

Effects of FM addition on oxidative damage in wheat. The overproduction of reactive oxygen species (ROS) generated by DEHP in plants might be responsible for disrupting cell membranes, inhibiting nutrient uptake, and enhancing lipid peroxidation. In this study, BC (FM) treatment decreased the content of O_2^- in wheat by 4.30–12.31% (7.39–23.13%) at the seedling stage and 1.05–10.06% (1.84–25.20%) at the booting stage (Figure 3A). BC (FM) application also induced a decrease in H_2O_2 content in wheat by 8.40–17.90% (10.10–30.00%) and 4.90–31.30% (12.40–25.20%) at the seedling and booting stage, respectively (Figure 3B). These results indicated that the application of BC and FM alleviated the oxidative damage in wheat caused by DEHP. Ma et al. (2019) also found that the addition of BC reduced the production of O_2^- in *Ipomoea aquatica* Forsk under dibutyl phthalate (DBP) stress due to a decrease in the accumulation of DBP in wheat roots and leaves, which is consistent with our results.

Plants exhibit their oxidative defence mechanisms to detoxify DEHP-induced reactive oxygen species and reduce the cascade of structural damage. This typically relies on the activation of different antioxidant enzymes and non-enzymatic antioxidants to scavenge reactive oxygen species. Our results showed that the activities of SOD and APX in wheat under DEHP stress were significantly enhanced by FM and BC in a dose-dependent manner ($P < 0.05$, Figure 4). After DEHP-contaminated soil amendment with 0.5–2% BC (FM), CAT activity in wheat leaves and roots increased by 9.10–15.6% (10.9–29.9%) and 6.66–21.3% (10.5–33.0%) at seedling stages, respectively, and 9.35–18.3% (7.32–30.4%) and 2.73–20.6% (8.47–29.3%) at booting stages, respectively, compared to that of the control. Mehmood et al. (2020) reported that chitosan-modified BC improved the activity of antioxidant enzymes, thereby reducing the accumulation of reactive oxygen species and improving the salt stress resistance of soybeans. Plants in DEHP-contaminated control soils without BC and FM possessed lower antioxidant activity and higher levels of oxidative stress, resulting in lower biomass. Therefore, we speculate that BC and FM reduced the exposure of wheat roots to DEHP because of their

porous structure and adsorption mechanisms, while they also induced antioxidant enzyme activity and decreased oxidative stress, which both contributed to improved growth and yield. These results correspond with this of previous investigations (Naeem et al. 2020).

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