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Co-application of biochar and melatonin enhances pea (*Pisum sativum* L.) performance and alleviates cadmium contamination stress

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Abstract: Sole biochar addition or exogenous melatonin application can decrease cadmium (Cd) toxicity in polluted soils and improve plant performance and growth. Yet the additive effects of biochar and melatonin application on plant growth, oxidative stress modulation and Cd absorption remain unclear. We conducted a pot experiment to study the combined effects of melatonin, biochar and Cd stress on pea (*Pisum sativum* L.) seedling growth, antioxidant enzyme activities, photosynthesis parameters and Cd uptake. Results showed that Cd addition significantly decreased pea growth, chlorophyll content, net photosynthesis rate (P_n), transpiration rate (T_r), stomatal conductance (g_s), and increased intercellular CO_2 concentration (c_i) and oxidant enzyme activities when compared to non-Cd contaminated treatment. Exogenous applications of the biochar or melatonin alone significantly decreased the harmful effects of Cd stress and promoted pea seedling growth. Moreover, soil remediation with biochar could more effectively improve pea growth, chlorophyll contents, and photosynthesis parameters and contribute to Cd immobilisation; the melatonin treatment alone could more effectively increase antioxidant enzyme activities. The treatments of biochar and melatonin showed an additive result and had the largest promoting in pea growth, antioxidant enzyme activities, and lowest Cd contents in pea tissue and soil. These results indicate that the combined use of melatonin and biochar is more effective at reducing Cd uptake by pea tissues and alleviating Cd harm to pea plants.

Keywords: heavy metal; transfer factor; fresh weight; soil quality; toxic element

Cadmium (Cd) is a classically deleterious heavy metal that contaminates agroecosystems. The main sources of Cd accumulation in soil are the overuse of pesticides and fertilisers, mining, smelting, and chemical manufacturing (Tang et al. 2020). Because of Cd's mobility in soil and solubility in water, plants readily absorb it. Cd has toxic effects on plant growth and threatens animal and mankind's health through the food chain (Rehman et al. 2020). The remediation of Cd-contaminated soil is critical, and it has attracted many scientists to participate in the research. Some studies have paid attention to removing or immobilising heavy metals in soil and improving plant tolerance to heavy metal stress by application of exogenous plant growth regulators and inorganic additives (Tousi et al. 2020, Mehdizadeh et al. 2021).

Biochar is the product of biological materials imperfect combustion in an oxygen-free environment, and has the properties of alkaline pH, micropores structure and large surface area. Biochar has been

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used in soil contaminated with heavy metals to decrease the harmful impacts on plant growth, human health, and environmental contamination (Liu et al. 2018). Melatonin (N-acetyl-5-methoxytryptamine) is a naturally occurring indoleamine that can regulate diverse physiological functions such as photoperiod, development, and growth of plants. Melatonin is also known as an abiotic stress modulator that can alleviate stress and improve plant performance. Previous research reported that melatonin application increased plant resistance to abiotic stress by reducing lipid peroxidation and hydrogen peroxide contents, alleviating the production of reactive oxygen species (ROS), and enhancing the antioxidant enzyme activities (Wu et al. 2021).

Some literature has reported on the combined application of biochar, other organic materials, and microbial inoculation in heavy metal-contaminated soil. Results showed that there were synergistic effects on regulating heavy metal adsorption, stabilisation, and soil quality, then improved plant performance (Yuan et al. 2019). Pea (*Pisum sativum* L.) is an important leguminous vegetable crop grown in temperate and semitropical regions, whose growth and yield are affected by Cd stress (Borah and Devi 2012). The influence of biochar and melatonin co-application on Cd remediation and pea performance has rarely been reported. In this study, we set a pot experiment to study the effects of melatonin and biochar, singly or in combination, on pea growth, photosynthesis parameters, antioxidant enzyme activities, and Cd uptake at different levels of Cd-contaminated soil. The aim of this research was to investigate the additive effects of co-application of biochar and melatonin on pea growth and Cd remediation.

MATERIAL AND METHODS

Melatonin, biochar and soil preparation. According to previous research, the melatonin concentration used in the study was 100 µmol/L for foliar spraying (Li et al. 2019). The biochar was derived from maize straw under high temperatures and in an oxygen-free environment. The biochar properties were: pH 10.5, 5.6 g/kg total nitrogen, 0.88 g/kg total phosphorus, 22.5 g/kg total potassium, and 4.68 g/kg organic carbon. The soil was sampled from an agricultural field in Luoyang, China (34.633°N, 112.467°E) and sifted through a 5-mm sieve. The soil was classified as Calcic Luvisols, and the soil physicochemical parameters were: 18.79% clay, 48.83% sand, 32.38% silt, 7.60 pH, 6.9 g/kg organic carbon, 45.93 mg/kg available nitrogen, 15.78 mg/kg available phosphorus, 105.33 mg/kg available potassium and 0.04 mg/kg total Cd.

Experimental design. This present study used 3 levels of Cd contamination (0, 3, and 6 mg/kg dissolved $CdCl_2$) to simulate soil with no slight and moderate Cd stress (SEPA 2018). Each Cd contamination level had four treatments: control (CK, without any intervention); amendment with biochar 40 g/kg soil (B); foliar spraying of exogenous melatonin with 100 µmol/L (M), and B in combination with M (BM). A pot experiment was conducted with six replicates of each treatment in the greenhouse of Henan University of Science and Technology, China. Each plastic pot (21 cm diameter × 27 cm depth) was filled with 5 kg of air-dried soil or a whole mixture of soil and biochar. Cd was added to the soil and mixed thoroughly using a $CdCl_2$ solution. Pea seeds (*Pisum sativum* cv. Zhongwan No. 6) were sterilised with 10% (*v*/*v*) hydrogen peroxide. Eight seeds were planted per pot on April 20, 2021, and four plants remained after germination. The melatonin was foliar sprayed for each M or BM treatment and deionised water for each CK or B treatment in the dark at 4-day intervals after 2 weeks of acclimatisation. No additional nutrients were applied during the experiment. After growing for 35 days, three replicates per treatment were used for measuring photosynthesis parameters, malondialdehyde (MDA) content, antioxidant enzymatic activities, and lipid peroxidation. The other three replicates were harvested to measure stem, leaf, and root weight, as well as Cd concentration in pea tissues. Rhizosphere soil samples were also harvested to analyse the soil's available Cd contents.

Measurements of chlorophyll content and photosynthesis parameters. The chlorophyll content index of SPAD (soil plant analysis development) value was determined with a portable chlorophyll meter (SPAD-502 Plus, Konica Minolta, Lincoln, Japan). The photosynthesis parameters, which include net photosynthetic rate (P_n), stomatal conductance (g_s), intercellular CO_2 concentration (c_i) and transpiration rate (T_r), were recorded by using Li-6400 portable photosynthesis meter (LI-COR, USA).

Determination of malondialdehyde, hydrogen peroxide and antioxidant enzymatic activities. The MDA content was evaluated according to Buege and Aust's procedure (1978). Hydrogen peroxide (H_2O_2) content was determined using titanium tetrachloride

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Table 1. Root, stem and leaf fresh weight of pea cultivated under cadmium stress

Cadmium concentration (mg/kg)	Treatment	Fresh weight of the root	Fresh weight of the stem	Fresh weight of the leaf
			(g/plant)	
0	CK	3.41 ± 0.10^{c}	2.78 ± 0.30^{c}	3.10 ± 0.05^{c}
	M	3.96 ± 0.26^{b}	3.45 ± 0.24^{b}	3.47 ± 0.20^{b}
	B	4.09 ± 0.11^{b}	3.63 ± 0.11^{b}	3.61 ± 0.14^{b}
	BM	4.51 ± 0.26^{a}	4.50 ± 0.27^{a}	4.19 ± 0.31^{a}
3	CK	3.11 ± 0.20^{c}	2.59 ± 0.25^{d}	2.92 ± 0.15^{c}
	M	3.52 ± 0.26^{b}	3.21 ± 0.27^{c}	3.22 ± 0.21^{b}
	B	3.69 ± 0.13^{b}	3.43 ± 0.18^{b}	3.40 ± 0.17^{b}
	BM	4.04 ± 0.23^{a}	4.11 ± 0.29^{a}	4.12 ± 0.29^{a}
6	CK	2.72 ± 0.35^{c}	2.42 ± 0.11^{c}	2.88 ± 0.07^{d}
	M	2.92 ± 0.22^{b}	2.92 ± 0.22^{b}	3.10 ± 0.10^{c}
	B	3.25 ± 0.09^{ab}	2.95 ± 0.19^{b}	3.30 ± 0.15^{b}
	BM	3.40 ± 0.11^{a}	3.27 ± 0.12^{a}	3.78 ± 0.18^{a}

Values are means ± standard deviation of three replicates; different letters indicate significant differences according to Duncan's multiple range test at $P < 0.05$. CK – control; B – biochar; M – melatonin; BM – biochar and melatonin

(Patterson et al. 1984). The nitroblue tetrazolium (NBT) photochemical reduction method was used to determine superoxide dismutase (SOD) activity (Rao and Sresty 2000). Peroxidase (POD) activity was determined by the guaiacol method, according to Ghosh and Singh (2005). Catalase (CAT) activity was measured by spectrophotometry, as described in Cakmak and Marschner (1992).

Determination of Cd content in soil and pea stem, leaf and root. Rhizosphere soil samples were collected after harvest by gently shaking pea roots by hand and air drying and sifting through a 5-mm sieve. Pea's stem, leaf and root tissues were ground and digested with 10 mL HNO_3-$HClO_4$ (3:1 *v*/*v*), then 5 mL of HNO_3 was added after overnight retention as described in Ryan et al. (2001). The Cd contents in pea and rhizosphere soil were measured using ICP-AES.

Calculation of bioconcentration factor and transfer factor. Cd's bioconcentration factor (BCF) was calculated as:

$$BCF = Cd_{aboveground\ concentration}/Cd_{soil\ concentration}.$$

Cd's transfer factor (TF) was calculated as:

$$TF = Cd_{aboveground\ concentration}/Cd_{root\ concentration}.$$

Statistical analysis. One-way ANOVA was performed using SPSS 22.0 (Armonk, USA), and Duncan's multiple range test was used to determine the significance among different treatments, taking $P < 0.05$ as significant. Origin 10.0 (Northampton, USA) drew all bar graphs.

RESULTS AND DISCUSSION

The root, stem, and leaf are of fresh weight to the pea. The growth traits of the fresh weight (FW) of the root, stem and leaf reduced when exposed to more Cd stress (Table 1). Compared with the CK treatment, B, M and BM treatments significantly improved pea growth ($P < 0.05$) at three Cd concentrations. The highest plant FW of leaf, stem and root were showed at the BM treatment without Cd stress. At 6 mg/kg Cd contamination level, compared with the CK, BM treat-

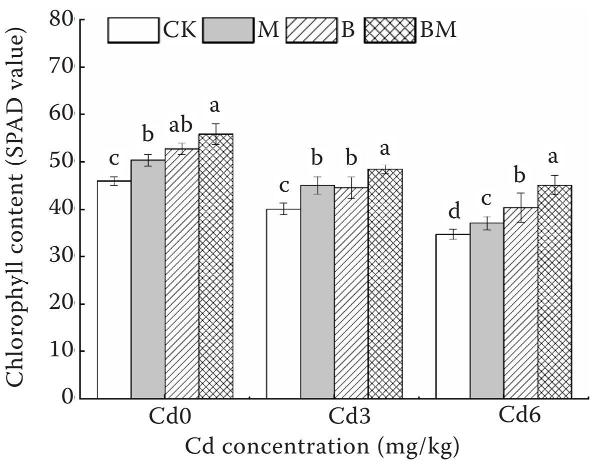

Figure 1. Chlorophyll content of pea cultivated under cadmium (Cd) stress. CK – control; B – biochar; M – melatonin; BM – biochar and melatonin; Cd0 – 0; Cd3 – 3; Cd6 – 6 mg/kg dissolved $CdCl_2$; SPAD – soil plant analysis development

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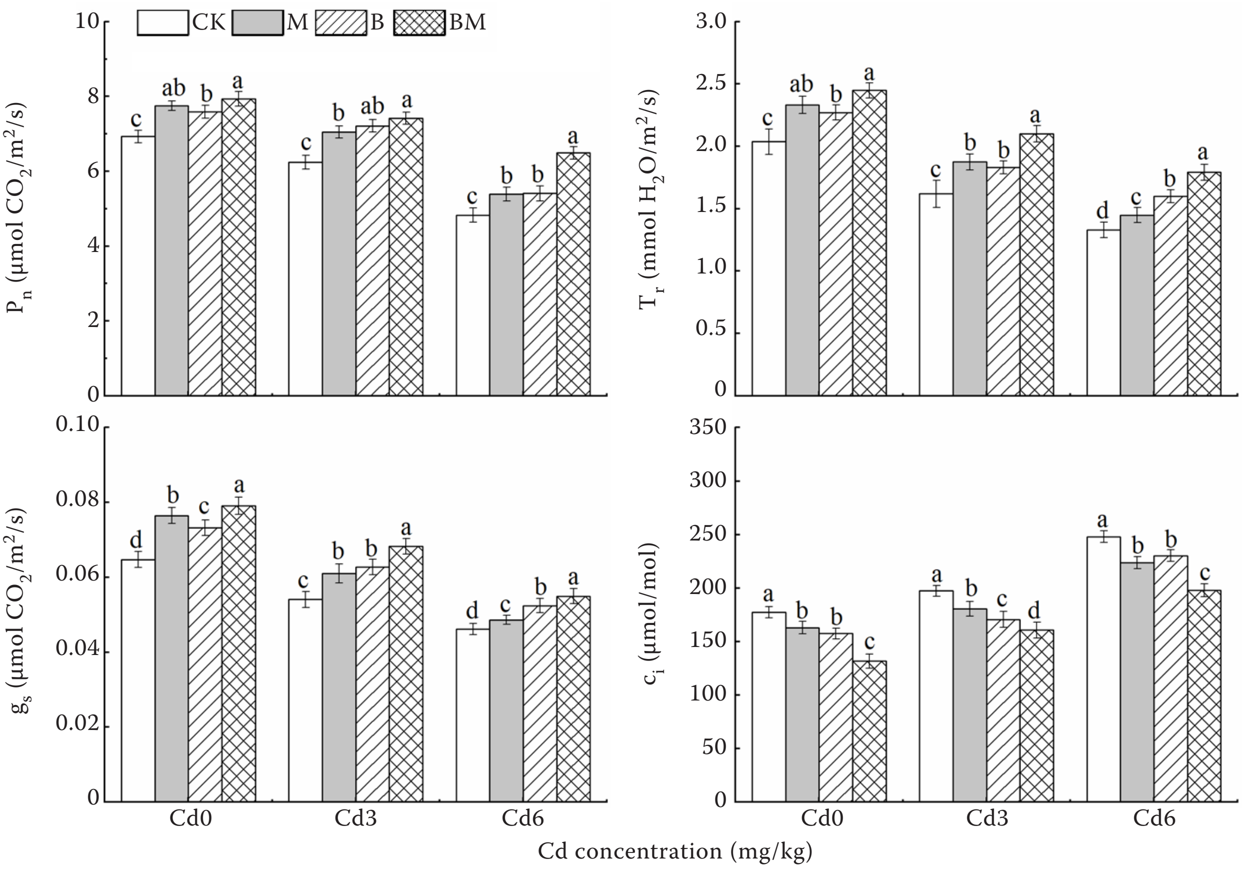

Figure 2. The net photosynthesis rate (P_n); transpiration rate (T_r); stomatal conductance (g_s) and intercellular CO_2 concentration (c_i) of pea cultivated under cadmium (Cd) stress. CK – control; B – biochar; M – melatonin; BM – biochar and melatonin; Cd0 – 0; Cd3 – 3; Cd6 – 6 mg/kg dissolved $CdCl_2$

ment increased FW of the root, stem and leaf by 25.02, 35.12 and 31.25%, respectively (Table 1). Furthermore, B was more effective than M, and the combination of B and M showed the best effect on increasing pea growth.

Chlorophyll content in pea leaves. With the aggravation of Cd stress, chlorophyll content showed a trend of decreasing (Figure 1). Compared with the CK, the treatments of B, M and BM all significantly ($P < 0.05$) increased chlorophyll contents. Under Cd0 and Cd3 stress levels, there were no significant differences ($P > 0.05$) between M and B in chlorophyll contents. However, under Cd6 stress, B was more effective than M in increasing chlorophyll contents. Notably, the combined application of B and M produced the best effects, which increased the SPAD values by 21.55, 20.98 and 29.87% under three Cd stress levels, respectively, compared to the CK.

Photosynthesis parameters. The photosynthesis parameters were significantly inhibited by Cd stress. The highest reduction in P_n, T_r and g_s was observed in Cd6 stress. The application of M, B and BM significantly ($P < 0.05$) increased the P_n, T_r and g_s values at three Cd stress conditions, and the combination of M and B showed the most significant effect. There was no significant difference ($P > 0.05$) between M and B in increasing P_n (Figure 2). The c_i values all significantly ($P < 0.05$) decreased under the B, M and BM treatments. Compared to the CK, the c_i values were reduced by 25.80, 17.05 and 20.11% under BM treatment at three Cd levels, respectively. These results suggested that combining B and M produced the best effect on alleviating Cd destruction to photosynthetic apparatus, followed by B and M treatments.

Oxidant and antioxidant enzymes activities. The highest contents of MDA and H_2O_2 were observed in Cd6 stress without B and M addition. The H_2O_2 and MDA contents significantly decreased with M, B and BM treatment under three Cd stress levels. The BM treatment showed the best effect of eliminating ROS (Figure 3). Compared with the CK, BM treatment decreased MDA contents by 52.22, 44.42 and 51.14%, decreased H_2O_2 contents by 31.57, 31.93 and 40.11%, respectively, under three Cd stress levels.

The SOD, POD and CAT activities were increased first and then decreased with the aggravation of Cd

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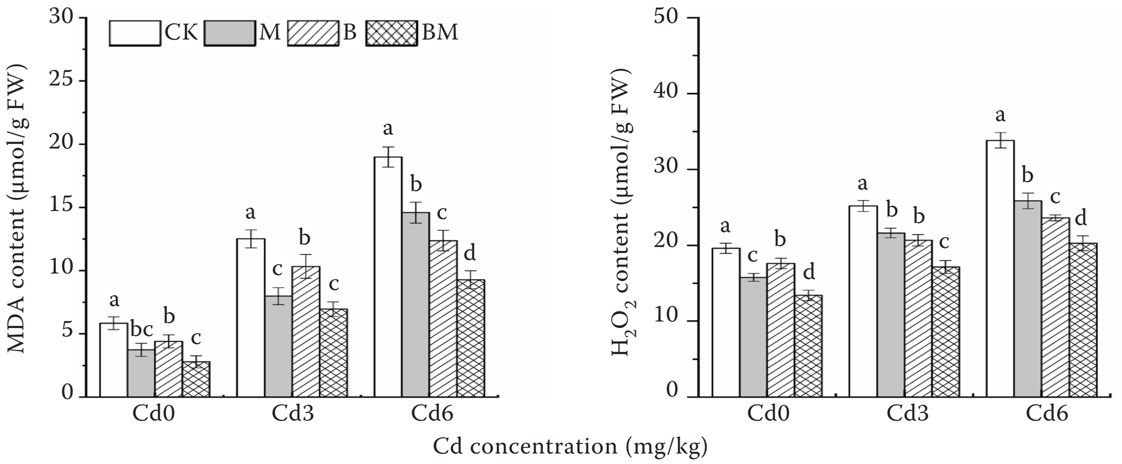
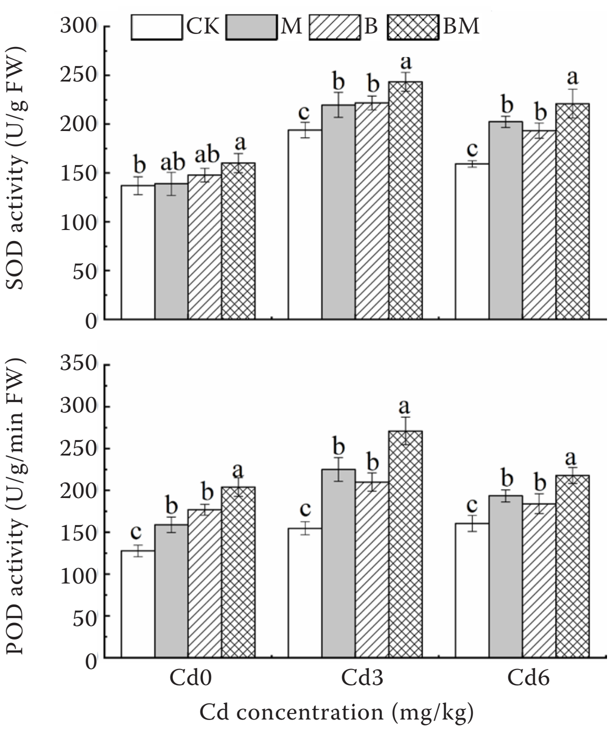


Figure 3. The malondialdehyde (MDA) and hydrogen peroxide (H_2O_2) contents of peas cultivated under cadmium (Cd) stress. CK – control; B – biochar; M – melatonin; BM – biochar and melatonin; FW – fresh weight; Cd0 – 0; Cd3 – 3; Cd6 – 6 mg/kg dissolved $CdCl_2$

contamination (Figure 4). Compared with the CK, the M, B and BM treatments all significantly ($P < 0.05$) increased the activities of SOD, POD and CAT under Cd3 and Cd6 contamination. For SOD and POD activities, there was no significant difference between M and B treatment under Cd0, Cd3 and Cd6 levels. Under three Cd levels, compared with the CK, the combination of B and M increased the SOD activity by 16.89, 25.48 and 38.72%, respectively, increased the POD activity by 59.74, 75.17 and 35.84%, respectively. Under Cd0 and Cd6 levels, there was no significant difference in CAT activity between M and B treatments. Under the Cd3 level, M significantly improved CAT activity more than B treatment. Compared with the CK, combining B and M increased the CAT activity by 71.40, 93.27 and 50.84%, respectively, under three Cd levels. The results showed that BM treatment had the strongest effects on improving antioxidant enzyme activities under Cd stress.

Cadmium concentration in soil and pea tissues. Cd concentration in pea tissues and soil increased with the aggravation of Cd stress levels (Figure 5).



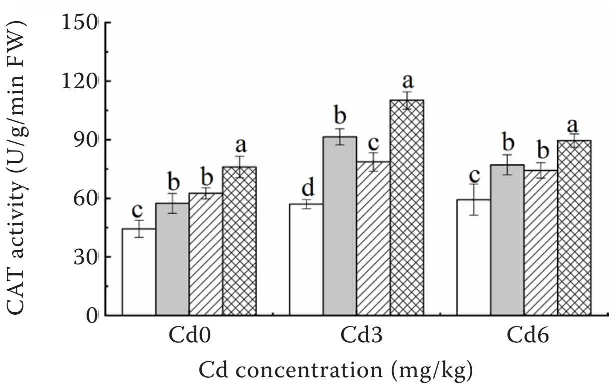


Figure 4. The antioxidant enzyme activities of pea cultivated under cadmium (Cd) stress. SOD – superoxide dismutase; CAT – catalase; POD – peroxidase; CK – control; B – biochar; M – melatonin; BM – biochar and melatonin; Cd0 – 0; Cd3 – 3; Cd6 – 6 mg/kg dissolved $CdCl_2$

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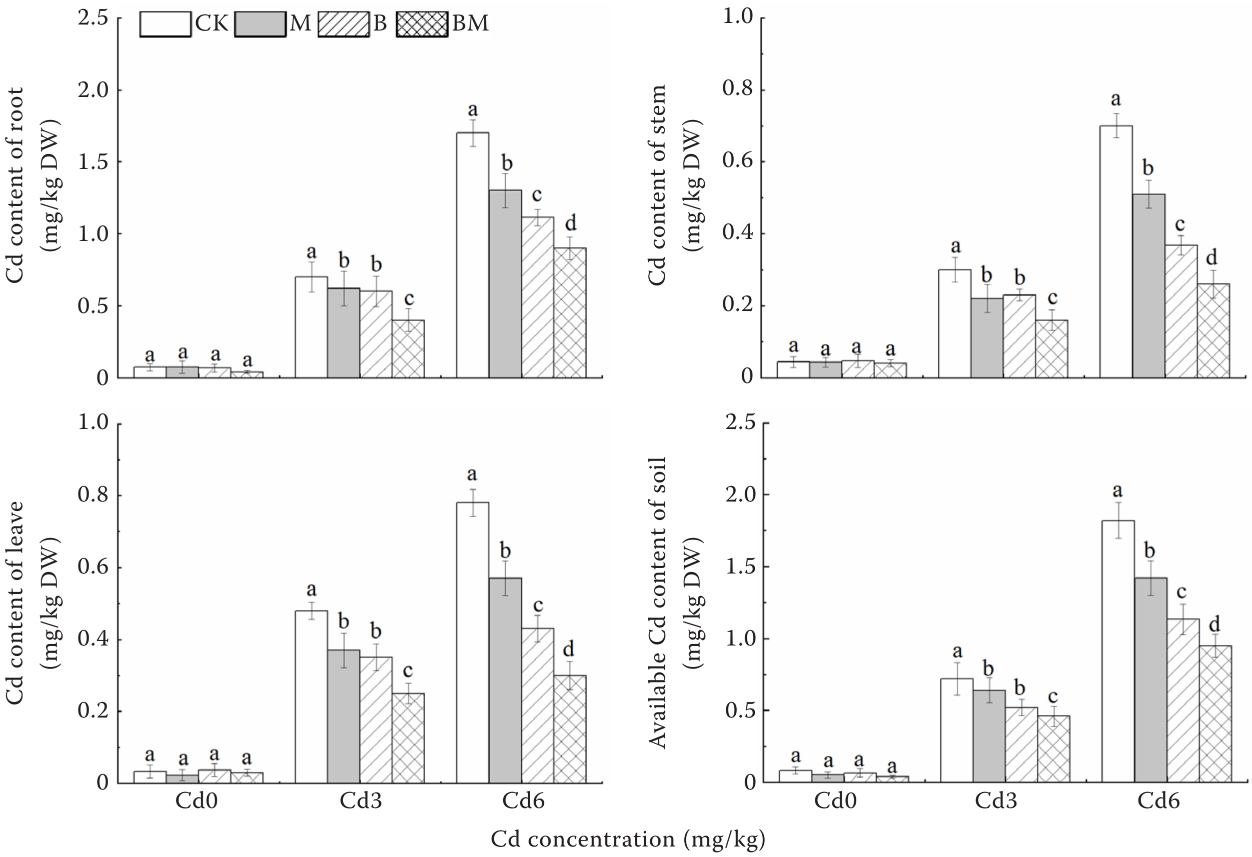

Figure 5. Cadmium (Cd) concentrations in soil, root, stem and leaves of peas cultivated under Cd stress. CK – control; B – biochar; M – melatonin; BM – biochar and melatonin; Cd0 – 0; Cd3 – 3; Cd6 – 6 mg/kg dissolved $CdCl_2$; DW – dry weight

Compared to the CK, Cd accumulation in pea tissues was decreased with B, M and BM treatments under Cd3 and Cd6 contamination levels (P < 0.05). The combined application of B and M showed the most significant reduction in Cd contents in pea tissues. Compared with the CK, Cd concentrations in root, stem and leaves were decreased by 42.85, 46.84 and 48.93% at the Cd3 stress level and reduced by 46.25, 59.02 and 59.53% at the Cd6 stress level, respectively. The available Cd content in soil was higher than in pea root, stem and leave. The BM treatment produced the greatest effect on reducing available Cd contents of the soil. These results indicate that BM had the best effect, and B had a greater effect than M on lowering pea tissues and soil Cd pollution.

Bioconcentration factor and transfer factor. The bioconcentration factor (BCF) and transfer factor (TF) of Cd were significantly (P < 0.05) reduced through B, M and BM amendments (Figure 6). The B, M and BM decreased the average BCF values under three Cd stress levels by 28.04, 40.98 and 54.48, respectively, and decreased the average TF values under three Cd stress levels by 10.03, 17.52 and 27.34%, respectively. These results showed that B was better than M in reducing BCF and TF values, and the combination of B and M was optimal.

DISCUSSION

The current study observed that the biomass of peas was increased, and Cd concentrations in pea tissues were reduced by biochar addition. There are several reasons why biochar decreases Cd damage in peas. Firstly, biochar contains valuable macronutrients and some beneficial metal ions, which increase soil fertility and promote pea growth. Secondly, the biochar derived from maize straw is alkaline, and the biochar addition might increase soil pH. The increased soil pH would change more Cd to the form of less mobile ions (Novak et al. 2009). This research showed that B treatment reduced the available Cd contents of the soil (Figure 5). Thirdly, biochar alleviated Cd toxicity in peas due to its physical properties of porous structure and large surface area, all of which enhance the detrimental ions absorption, soil water holding capacity and soil

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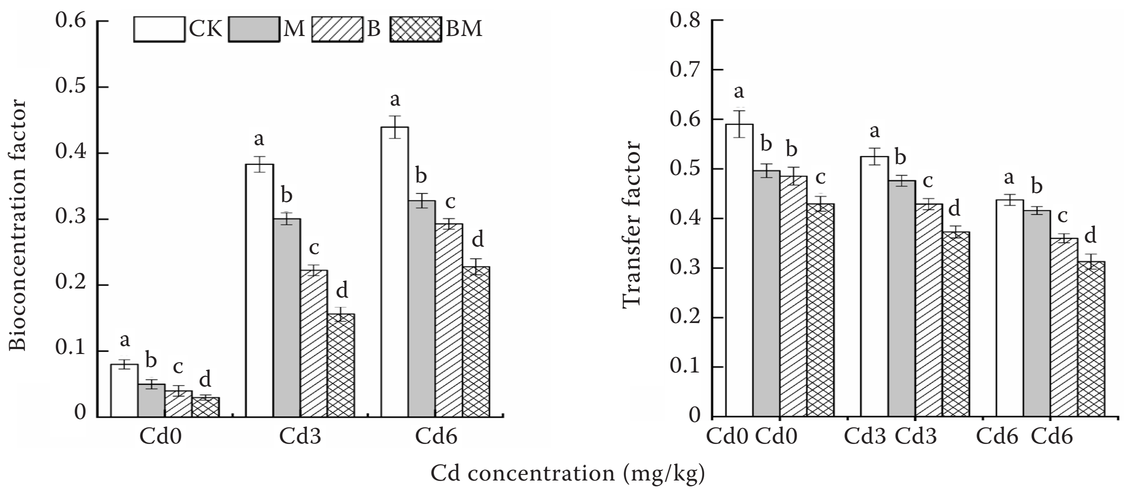

Figure 6. The bioconcentration factor and transfer factor of peas cultivated under cadmium (Cd) stress. CK – control; B – biochar; M – melatonin; BM – biochar and melatonin; Cd0 – 0; Cd3 – 3; Cd6 – 6 mg/kg dissolved $CdCl_2$

moisture availability (Khan et al. 2020). The increased water holding capacity and moisture availability can dilute ions in the soil, reducing ions toxicity to plants. In our study, chlorophyll content and photosynthetic capacity increased by B application, which reflected Cd toxicity alleviation. It was reported that biochar alleviated Cd-induced oxidative stress in wheat plants by decreasing MDA and H_2O_2 concentration (Abbas et al. 2018). Our results also showed that MDA and H_2O_2 concentration decreased, while SOD, POD, and CAT activities increased under B application (Figure 3). The mechanisms of melatonin application in alleviating Cd stress are as follows. Firstly, melatonin plays a role as a ROS scavenger that protects against Cd stress. The addition of melatonin to reduce oxidative damage caused by heavy metal stress through eliminating ROS and enhancing the antioxidative enzyme activities has been documented in previous studies (Park et al. 2013, Kaya et al. 2019). The research showed that M treatments decreased the Cd-induced oxidative stress indicators, such as H_2O_2 and MDA contents, and increased the antioxidant enzyme activities (Figure 4). Secondly, melatonin may preserve chlorophyll content by down-regulating chlorophyllase expression and up-regulating stress tolerance-related genes (Liang et al. 2019). In this study, M treatments improved peas's chlorophyll content and photosynthetic capacity under Cd stress. Consistent with our view, some previous studies have also reported melatonin's effect on promoting the plant's photosynthetic capacity (Xu et al. 2017, Liang et al. 2019). Thirdly, melatonin plays an important role in reducing the translocation of Cd from the soil to the aboveground parts of pea plants. The reduced Cd translocation from the soil is an effective strategy to decrease the phytotoxicity of Cd to the photosynthetic apparatus.

In the present study, B, in combination with M, adapted pea to Cd stress more effectively, with the highest antioxidant enzyme activities, chlorophyll contents and lowest Cd concentration in pea tissues. This indicates an additive effect between B and M. Farouk and Al-Huqail (2022) showed that the combination application of biochar and melatonin had the best effect for improving salinity tolerance in borage plants. Alharby and Fahad (2020) also suggested that melatonin application can enhance the efficiency of biochar in inducing drought tolerance in plants. In this study, the treatments of BM ameliorated leaf gas exchange traits under Cd stress (Figure 2). Our findings are further confirmed by Alharby and Fahad (2020), who showed that exogenous application of melatonin and biochar assuaged the adverse effects of drought stress on all leaf gas exchange traits. These results showed that BM had the best effect in reducing Cd translocation from the soil to pea tissue and strengthening the contribution to Cd immobilisation. Furthermore, the detailed mechanisms of the combined effect of B and M need further investigation.

In summary, exogenous application of B and/or M can lighten the Cd damage on pea as well as reduce Cd uptake in pea tissues. The B amendment was more effective at improving pea growth, chlorophyll contents, and photosynthesis parameters and contributing to Cd immobilisation in soil. The M treatment

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was more effective at improving antioxidant enzyme activities. The combination of B and M treatments showed an additive effect and had the largest alleviation of Cd stress and increased pea growth.

REFERENCES

Abbas T., Rizwan M., Ali S., Adrees M., Zia-ur-Rehman M., Qayyum M.F., Ok Y.S., Murtaza G. (2018): Effect of biochar on alleviation of cadmium toxicity in wheat (*Triticum aestivum* L.) grown on Cd-contaminated saline soil. Environmental Science and Pollution Research, 25: 25668–25680.

Alharby H.F., Fahad S. (2020): Melatonin application enhances biochar efficiency for drought tolerance in maize varieties: modifications in physio-biochemical machinery. Agronomy Journal, 112: 2826–2847.

Borah M., Devi A. (2012): Effect of heavy metals on *Pisum sativum* Linn. International Journal of Advanced Biological Research, 2: 314–321.

Buege J.A., Aust S.D. (1978): Microsomal lipid peroxidation. Methods in Enzymology, 52: 302–310.

Cakmak I., Marschner H. (1992): Magnesium deficiency and high light intensity enhance activities of superoxide dismutase, ascorbate peroxidase, and glutathione reductase in bean leaves. Plant Physiology, 98: 1222–1227.

Farouk S., Al-Huqail A.A. (2022): Sustainable biochar and/or melatonin improve salinity tolerance in borage plants by modulating osmotic adjustment, antioxidants, and ion homeostasis. Plants-Basel, 11: 765.

Ghosh M., Singh S.P. (2005): A comparative study of cadmium phytoextraction by accumulator and weed species. Environmental Pollution, 133: 365–371.

Kaya C., Okant M., Ugurlar F., Alyemeni M.N., Ashraf M., Ahmad P. (2019): Melatonin-mediated nitric oxide improves tolerance to cadmium toxicity by reducing oxidative stress in wheat plants. Chemosphere, 225: 627–638.

Khan A.Z., Ding X.D., Khan S., Ayaz T., Fidel R., Khan M.A. (2020): Biochar efficacy for reducing heavy metals uptake by cilantro (*Coriandrum sativum*) and spinach (*Spinacia oleracea*) to minimize human health risk. Chemosphere, 244: 125543–125585.

Li D., Wang Y.F., Wang Y.H., Wen X.L., Cai H.Y., Zheng X.L., Chen T.T., Liu L. (2019): Effects of exogenous melatonin on seed germination, seedling resistance physiological and Cd content of pea under cadmium stress. Journal of Nuclear Agricultural Sciences, 33: 2271–2279. (In Chinese)

Liang D., Ni Z.Y., Xia H., Xie Y., Lv X.L., Wang J., Lin L.J., Deng Q.X., Luo X. (2019): Exogenous melatonin promotes biomass accumulation and photosynthesis of kiwifruit seedlings under drought stress. Scientia Horticulturae, 246: 34–43.

Liu L., Li J.W., Yue F.X., Yan X.W., Wang F.Y., Bloszies S., Wang Y.F. (2018): Effects of arbuscular mycorrhizal inoculation and biochar amendment on maize growth, cadmium uptake and soil cadmium speciation in Cd-contaminated soil. Chemosphere, 194: 495–503.

Mehdizadeh L., Farsaraei S., Moghaddam M. (2021): Biochar application modified growth and physiological parameters of *Ocimum ciliatum* L. and reduced human risk assessment under cadmium stress. Journal of Hazardous Materials, 409: 124954.

Novak J.M., Busscher W.J., Laird D.L., Ahmedna M., Watts D.W., Niandou M.A.S. (2009): Impact of biochar amendment on fertility of a southeastern coastal plain soil. Soil Science, 174: 105–112.

Park S., Lee D.E., Jang H., Byeon Y., Kim Y.S., Back K. (2013): Melatonin-rich transgenic rice plants exhibit resistance to herbicide-induced oxidative stress. Journal of Pineal Research, 54: 258–263.

Patterson B.D., Macrae E.A., Ferguson I.B. (1984): Estimation of hydrogen peroxide in plant extracts using titanium (IV). Analytical Biochemistry, 139: 487–492.

Rao K.V.M., Sresty T.V.S. (2000): Antioxidative parameters in the seedlings of pigeonpea (*Cajanus cajan* (L.) Millspaugh) in response to Zn and Ni stresses. Plant Science, 157: 113–128.

Rehman M.Z.U., Batool Z., Ayub M.A., Hussaini K.M., Murtaza G., Usman M., Naeem A., Khalid H., Rizwan M., Ali S. (2020): Effect of acidified biochar on bioaccumulation of cadmium (Cd) and rice growth in contaminated soil. Environmental Technology and Innovation, 19: 101015.

Ryan P.R., Delhaize E., Jones D.J. (2001): Function and mechanism of organic anion exudation from plant roots. Annual Review of Plant Physiology and Plant Molecular Biology, 52: 527–560.

SEPA (2018): Soil Environmental Quality-Risk Control Standard for Soil Contamination of Agricultural Land. China, State Environmental Protection Administration, GB15618–2018.

Tang J.Y., Zhang L.H., Zhang J.C., Ren L.H., Zhou Y.Y., Zheng Y.Y., Lin L., Yuan Y., Huang H.L., Chen A.W. (2020): Physicochemical features, metal availability and enzyme activity in heavy metal-polluted soil remediated by biochar and compost. Science of the Total Environment, 701: 134751.

Tousi S., Zoufan P., Ghahfarrokhie A.R. (2020): Alleviation of cadmium-induced phytotoxicity and growth improvement by exogenous melatonin pretreatment in mallow (*Malva parviflora*) plants. Ecotoxicology and Environmental Safety, 206: 111403.

Wu S.Q., Wang Y., Zhang J.K., Gong X.J., Zhang Z., Sun J.J., Chen X.S., Wang Y.L. (2021): Exogenous melatonin improves physiological characteristics and promotes growth of straw berry seedlings under cadmium stress. Horticultural Plant Journal, 7: 13–22.

Xu L.L., Yue Q.Y., Bian F.E., Sun H., Zhai H., Yao Y.X. (2017): Melatonin enhances phenolics accumulation partially *via* ethylene signaling and resulted in high antioxidant capacity in grape berries. Frontiers in Plant Science, 8: 1426.

Yuan P., Wang J.Q., Pan Y.J., Shen B.X., Wu C.F. (2019). Review of biochar for the management of contaminated soil: preparation, application and prospect. Science of Total Environment, 659: 473490.

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