Journal of
Plant Ecology

Biochar and alternate partial root-zone irrigation greatly enhance the effectiveness of mulberry in remediating lead-contaminated soils

Lei Wang[1,2], Qing-Lai Dang[2,*] and Binyam Tedla[2]

[1]Department of Biotechnology, College of Biotechnology, Jiangsu University of Science and Technology, Jiangsu, China, [2]Department of Forestry, Faculty of Natural Resources Management, Lakehead University, Thunder Bay, Ontario P7B 5E1, Canada

*Corresponding author. E-mail: qdang@lakeheadu.ca

Handling Editor: Xiaojuan Feng

Received: 23 May 2020, **Revised:** 19 July 2020, **Accepted:** 14 September 2020**, Advanced Access publication:** 16 September 2020

Citation: Wang L, Dang Q-L, Tedla B (2020) Biochar and alternate partial root-zone irrigation greatly enhance the effectiveness of mulberry in remediating lead-contaminated soils. *J Plant Ecol* **13**:757–764. https://doi.org/10.1093/jpe/rtaa063

Abstract

Aims Soil lead contamination has become increasingly serious and phytoremediation can provide an effective way to reclaim the contaminated soils. This study aims to examine the growth, lead resistance and lead accumulation of mulberry (*Morus alba* L.) seedlings at four levels of soil lead contamination with or without biochar addition under normal or alternative partial root-zone irrigation (APRI).

Methods We conducted a three-factor greenhouse experiment with biochar (with vs. without biochar addition), irrigation method (APRI vs. normal irrigation) and four levels of soil lead (0, 50, 200 and 800 mg·kg^{-1}). The performance of the seedlings under different treatments was evaluated by measuring growth traits, osmotic substances, antioxidant enzymes and lead accumulation and translocation.

Important Findings The results reveal that mulberry had a strong ability to acclimate to soil lead contamination, and that biochar and APRI synergistically increased the biomass and surface area of absorption root across all levels of soil lead. The seedlings were able to resist the severe soil lead contamination (800 mg·kg^{-1} Pb) by adjusting glutathione metabolism, and enhancing the osmotic and oxidative regulating capacity via increasing proline content and the peroxidase activity. Lead ions in the seedlings were primarily concentrated in roots and exhibited a dose–effect associated with the lead concentration in the soil. Pb, biochar and ARPI interactively affected Pb concentrations in leaves and roots, translocation factor and bioconcentration. Our results suggest that planting mulberry trees in combination with biochar addition and APRI can be used to effectively remediate lead-contaminated soils.

Keywords: Pb-contaminated soil, phytoremediation, *Morus alba* L., alternate partial root-zone irrigation, biochar

摘要：土壤铅污染日益严重，植物修复是一种环保的污染土壤修复技术。本文旨在研究四种土壤铅污染水平下，添加生物碳和分根区交替灌溉(Alternative Partial Root-zone Irrigation，APRI)对桑树幼苗的生长、铅适应性和铅积累的影响。我们以生物碳(添加与不添加生物碳)、灌溉方式(APRI 与常规灌溉)和土壤铅水平(0、50、200 和800 mg kg^{-1} Pb)为三因素实施了温室试验。通过测定桑树幼苗的生长性状、渗透物质代谢、抗氧化酶活性、铅的积累和转运等参数，探讨了不同处理对桑树生长发育的影响。结果表明，桑树对土壤铅污染有较强的适应能力；生物碳和APRI 在不同土壤铅水平上协同提高了生物量和吸收根表面积。桑树通过调节谷胱甘肽 (GSH)、脯氨酸代谢和过氧化物酶(POD)活性，增加了渗透和抗氧化调节能力，进而提高了对重度铅污染土壤(800 mg kg^{-1})的抗性。桑苗中的铅离子主要集中在根中，与土壤铅浓度具有剂量效应。土壤铅、生物碳和ARPI的交互作用影响了叶片和根系中铅的浓度、转运和生物富集系数。综上所述，在桑树栽培中结合外源生物碳和APRI可有效地用于修复土壤铅污染。

关键词：土壤铅污染，植物修复技术，桑树，分根区交替灌溉，生物碳

INTRODUCTION

The rapid economic development and fast rate of urbanization have led to increased heavy metal pollution to soils in China (PRC Ministry of the Environment and the Ministry of Natural Resources 2014). A survey by the Ministry of the Environment and the Ministry of Natural Resources of China has revealed that the concentrations of pollutants in the soil exceed the national standards by an average of 16.1% at all the sample sites that cover all the arable lands and parts of forests, grassland, urban area and unused land, and by 19.4% on farmlands with heavy metals as the primary culprit at 82.8% of the sites (PRC Ministry of the Environment and the Ministry of Natural Resources 2014). The heavy metal pollutants come from a wide range of sources and are not biodegradable (Yang *et al.* 2018). Heavy metals in soils are highly toxic to animals, plants, microorganisms and human beings; their ions are highly stable and have poor mobility, resulting in an extended period of retention in the soil (Xinxin *et al.* 2020). Effective and economic means to reduce or eliminate heavy metal elements from the soil and to restore the productivity of the lands are urgently needed but are currently not available. Some plant species have high capacities to absorb and accumulate heavy metals and may represent a viable option for the remediation of heave metal contaminated soils (Gerhardt *et al.* 2017; Peng *et al.* 2020b; Ullah *et al.* 2015).

Lead is a common heavy metal contaminant in soils due to its wide range of sources and high solubility in water (Kumar and Prasad 2018). It has been found that lead concentration in the soil within 150 m on the sides of highways in China exceeds the national standard by 20.3% (PRC Ministry of the Environment and the Ministry of Natural Resources 2014). The electric vehicle industry has been booming in recent years in China, e.g. the ownership of electric bicycles reached 181 million in April 2014 and the production of lead-acid batteries in China has reached 1/3 of the total global output, which may cause further lead contamination from improper control of the batter production process and/or improper treatment of used batteries (Sun *et al.* 2016). Even low levels of lead can cause serious harm to plants, animals and humans. For example, lead ions can induce reactive oxygen species in plants, leading to physiological disorders, tissues and cell deformations, disruptions of growth and development, even death (Asgari Lajayer *et al.* 2017; Berni *et al.* 2019; Mahdavian *et al.* 2016). However, the effects may vary with species, development stages and other environmental conditions (Zhang *et al.* 2020). Finding plant species that can tolerate the high concentration of lead in the soil and also have the ability to remove it from the soil has become a high priority in the scientific community.

Mulberry (*Morus alba* L.) is an ecologically and economically important native tree species in China; it has a wide range of natural distribution and has been cultivated widely due to its ability to thrive over a wide range of ecological conditions and high economic values, such as sericulture (He *et al.* 2013; Ming *et al.* 2019). Mulberry can also be potentially used in ecosystem rehabilitation and water and soil conservation (Liu *et al.* 2016; Sánchez-Salcedo *et al.* 2015). Mulberry is known to tolerate some heavy metal elements, e.g. lead, cadmium, chromium and arsenic(Peng *et al.* 2020b; Zhang *et al.* 2020). The common practice of twice-a-year pruning in managing mulberry plantations can potentially provide an effective means to remove heavy metals from polluted sites (Wan *et al.* 2017; Zeng *et al.* 2020). However, it is unknown what level of lead contamination it can tolerate and how its growth and ability to accumulate lead in its biomass will change with the severity of lead pollution.

Alternate partial root-zone irrigation (APRI), also known as partial root-zone drying, is a novel technology where the plant root system is partitioned into two halves and only one half is irrigated or dried at a time and the irrigation alternates between the two halves (Dodd *et al.* 2015). APRI can enhance plant resistance to drought without compromising biomass production (Consoli *et al.* 2017; Ennahli *et al.* 2015). However, it is unknown whether APRI may increase plant resistance to heavy metals and/or improve the ability to remediate heavy metal polluted soils.

Biochar refers to porous solid materials that are produced via the pyrolysis of carbon-rich biomass under oxygen-limited conditions (Kavitha *et al.* 2018; Wang *et al.* 2018). Biochar is often used as a soil conditioner or pollutant adsorbent due to its high adsorption capacity, stability, safety, low cost, easy availability and environmental compatibility (Livne-Luzon *et al.* 2016) and can potentially be used alone or in combination with APRI to enhance the ability of plants to remediate contaminated soils. However, there is no information in the literature on the interactive effects of biochar, APRI and severity of soil pollution on the physiology, growth and heavy metal sequestration ability of plants (Tu *et al.* 2020). In this study, we used a greenhouse experiment to investigate the responses of mulberry to a wide range of soil Pb concentration and to examine the interactive effects of biochar, APRI and soil Pb concentrations on its growth and ability to accumulate and translocate Pb. We test the hypothesis that biochar and APRI will synergistically enhance the growth and lead resistance of mulberry. The results will provide a theoretical basis for the efficient use of mulberry as a phytoremediation species in the restoration of lead-contaminated soils.

MATERIALS AND METHODS

Materials and experimental design

The experiment was carried out in the greenhouse at the Jiangsu University of Science and Technology National Mulberry Resource Garden (Zhenjiang, China, 31°10′50″ N, 119°23′15″ E). The '*Fresh fruit No.1*' mulberry cultivar, a dual purpose of leaves and fruits cultivar with large biomass, was used as the plant material. The loam top-soil (top 5 cm) for the experiment was collected from the National Mulberry Resource Garden. The soil properties were 6.56 pH, 23.9 mg·kg^{-1} Pb, 357.3 mg·kg^{-1} total nitrogen (N), 9.1 mg·kg^{-1} available P and 78.24 mg·kg^{-1} available K.

103.5 g·kg^{-1} Pb treatment soil (referred as parent soil) was prepared as follows: dry loam soil was sifted through a 2-mm mesh sieve; 1668.8 g sifted soil was added to 1 l water solution containing 331.23 g $Pb(NO_3)_2$ and mixed thoroughly; the mixture was oven-dried for 48 h, ground, and sifted through a 10-mesh sieve.

The experiment was a completely randomized design with three factors (Table 1): lead stress (four levels), irrigation method (two levels: normal vs. APRI) and biochar application (two levels: with vs. without biochar addition). There were four replicates for each treatment. Two-year-old seedlings were transplanted into plastic pots (13 cm bottom diameter, 16 cm top diameter and 17.5 cm in height). The four levels of lead treatment were achieved by amending the soil with 0, 0.15, 0.6 and 2.4 g Pb parent soil to achieve 0, 50, 200 and 800 mg·kg^{-1} Pb concentrations, representing Pb-free, mild, moderate and severe soil Pb stress, respectively. The biochar treatment was achieved by adding biochar to 5% of the soil dry weight. The APRI treatment was implemented according to Wang *et al.* (2016), i.e. the root system was split equally into the two compartments during transplanting. The seedlings were allowed 2 months to acclimate before the irrigation treatments were initiated. Each seedling was weighed (with the container) every day and irrigated with 500 ml water two to four times a week according to the rate of weight loss to ensure that there was no water stress. For the normal irrigation treatment, the entire root system was watered. For the APRI treatment, only one of the two root compartments was watered each time and the irrigation was rotated between the two root compartments every 2 weeks. The water supply duration and volume were consistent for every seedling during the test period. The greenhouse was well-ventilated and the growth conditions were similar to those in the ambient environment except precipitation.

Table 1: Outline of experimental design and treatments

Treatment	Pb concentration (mg·kg^{-1} of soil DW)	Biochar concentration (percent of soil DW)	Irrigation regime
P_0	0	0	Normal
P_{50}	50	0	Normal
P_{200}	200	0	Normal
P_{800}	800	0	Normal
P_0A	0	0	APRI
P_{50}A	50	0	APRI
P_{200}A	200	0	APRI
P_{800}A	800	0	APRI
P_0B	0	5	Normal
P_{50}B	50	5	Normal
P_{200}B	200	5	Normal
P_{800}B	800	5	Normal
P_0AB	0	5	APRI
P_{50}AB	50	5	APRI
P_{200}AB	200	5	APRI
P_{800}AB	800	5	APRI

APRI: the root system was divided into two halves, only one half was irrigated every 2 weeks and the irrigation application was alternated between the two halves; Normal: the entire root system was irrigated every 2 weeks; DW: dry weight.

Growth traits and lead content measurements

After 3 months of treatment, four seedlings as replicates were randomly selected from each treatment combination for growth parameters measurements (biomass, root–shoot ratio, climax leaf area and absorption root surface area). The roots were rinsed with running water, and the climax leaf (one of 4th–6th leaf from the top) area and the total surface area of absorption roots (diameter ≤2 mm) were measured using an LA-S Plant Image Analysis System (Wseen Inc., Hangzhou, Zhejiang, China). The roots, stem and leaves were then oven-dried at 75 °C for 48 h and their dry masses were measured. 0.5 g oven-dried roots and 0.5 g leaves from each seedling were ground and digested in 10 ml $HNO_3/HClO_4$ solution using a microwave digestion system. Deionized water was added to the digested solution to a total volume of 25 ml. The lead content of the sample was determined using an Elan DRC-e inductively coupled plasma mass spectrometer (ICP-MS) (PerkinElmer Inc., Waltham, MA, USA) at Yangzhou University Test Center.

Osmotic substances and antioxidant enzymes measurements

For the determination of biochemical parameters, 0.5 g leaves from each treatment were sampled and immediately ground in a 2 ml plant extract solution using a prechilled mortar and centrifuged at 4000 rpm for 10 min at 4 °C using a cold centrifuge (Sigma Inc., USA). The supernatant was stored at 4 °C for further measurement. Proline content, soluble sugar content, superoxide dismutase (SOD) and peroxidase (POD) activity, glutathione (GSH) content and nonprotein

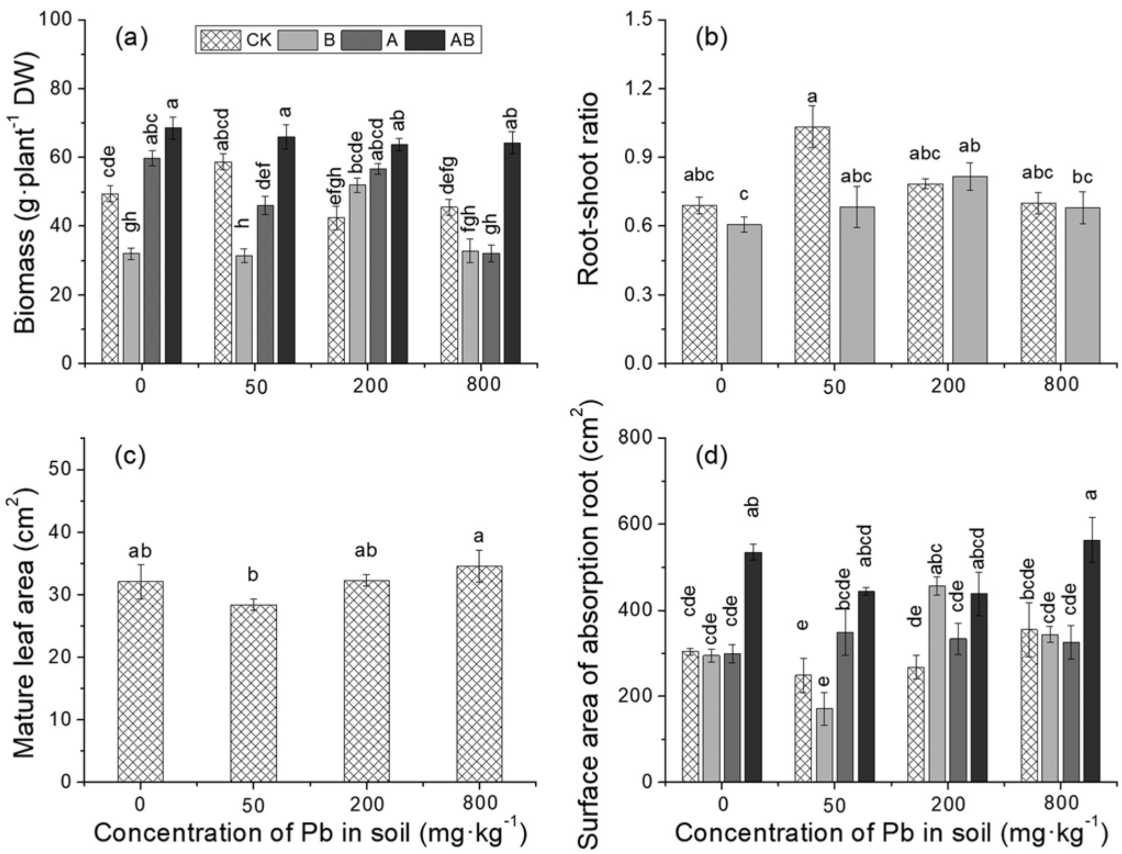

Figure 1: Effects of Pb concentration, irrigation method and biochar application on the biomass (**a**), root–shoot ratio (**b**), mature leaf area (**c**) and surface area (**d**) of absorption roots in mulberry seedlings after 3 months of treatments. CK = regular irrigation + no biochar, A = alternative irrigation + no biochar, B = regular irrigation + biochar, AB = alternative irrigation + biochar, DW = dry weight. Means (±1 SEM) with different letters were significantly different between each other ($P \leq 0.05$). DW: dry weight.

Table 2: ANOVA *P*-values for the effects of lead concentration (Pb), irrigation method and biochar application on growth traits, osmotic substances, antioxidant enzymes, lead accumulation and translocation in mulberry seedlings

Factors	Biomass	Root–shoot ratio	Climax leaf area	Absorption root surface area	Proline content	Soluble sugar content	POD activity	SOD activity	GSH content	NTP content	Pb in leaves	Pb in roots	Translocation factor	Bioconcentration factor
Pb	<0.001	0.042	0.008	0.007	0.001	0.108	<0.001	<0.001	0.001	0.168	<0.001	<0.001	<0.001	0.001
Irrigation	<0.001	0.044	<0.001	<0.001	<0.001	0.703	<0.001	0.006	<0.001	0.003	0.028	<0.001	<0.001	0.384
Biochar	0.052	0.02	0.805	<0.001	0.038	0.644	0.259	0.184	<0.001	0.004	<0.001	<0.001	0.381	<0.001
Pb × irrigation	0.002	0.191	0.589	0.026	<0.001	0.002	0.025	0.002	0.003	0.191	<0.001	<0.001	0.004	0.035
Pb × biochar	<0.001	0.011	0.747	0.058	0.74	0.436	0.197	0.013	0.107	0.14	<0.001	<0.001	0.002	0.005
Irrigation × biochar	<0.001	0.567	0.193	<0.001	0.719	<0.001	0.421	<0.001	<0.001	0.197	0.005	0.877	0.935	<0.001
Pb × irrigation × biochar	<0.001	0.223	0.362	0.007	0.045	0.772	0.003	0.192	0.079	0.67	<0.001	<0.001	<0.001	<0.001

thiol content were measured using test kits from Nanjing Jiancheng Bioengineering Institute (Nanjing, China). All the assays were performed according to the instructions.

Calculation of Pb translocation factor and bioconcentration factor

The Pb translocation factor (Tf) and bioconcentration factor (Bf) were calculated as follows:

$$\text{Translocation factor} = \text{leaf Pb content}/\text{root Pb content}$$

$$\text{Bioconcentration factor} = \text{seedling Pb content in seedling}/\text{substrate Pb content}$$

Statistical analysis

The data were analyzed using analysis of variance (ANOVA) with R v 3.6.1 (R Development Core Team 2019). Shapiro test and residual plots were conducted to test the ANOVA assumptions of normality and homogeneity, and power transformation was applied where the assumptions were not met. Fisher's least significant difference *post hoc* pairwise comparisons were conducted when ANOVA showed a significant interaction ($P \le 0.05$).

RESULTS

Growth traits

The combination of biochar addition and APRI generally yielded the highest biomass among all the treatment combinations, their individual effects on biomass varied with Pb concentrations (Fig. 1a and Table 2). ANOVA (Table 2) showed a significant interaction between Pb and biochar on root–shoot ratio, but no apparent response patterns were identified with the exception that biochar application tended to reduce root/shoot ratio when was no or very low concentration of Pb in the soil (Fig. 1b). The climax leaf area was significantly greater in the P_{800} than P_{50} treatment (Fig. 1c and Table 2). The combination of biochar application and APRI generally produced the largest absorption root area in all the Pb treatments and the effect was particularly large in P_0 and P_{800} (Fig. 1d). The maximum absorption root area (563.2 ± 52.6 cm^2) occurred at the highest Pb concentration (PAB_{800}) while the minimum absorption root surface area (171.5 ± 21.3 cm^2) occurred at the lowest Pb concentration (PB_{50}) (Fig. 1d).

Osmotic substances and antioxidant enzymes

Leaf proline concentration was generally the lowest in regular irrigation with no biochar in all the Pb treatments and the difference among irrigation method and biochar treatment was greatest at the highest Pb concentration (from 34.2 to 77.7 μg·g^{-1} fresh weight, Fig. 2a and Table 2).

The combination of APRI and 200 mg kg^{-1} Pb and that of regular irrigation and 800 mg kg^{-1} Pb resulted in the lowest soluble sugar concentration among all the irrigation and Pb combinations (Fig. 2b and Table 2).

Biochar addition significantly decreased soluble sugar concentration under regular irrigation but increased it under APRI; in contrast, APRI decreased soluble sugar concentration in absence of biochar but increased it in the presence of biochar (Fig. 2c). APRI led to the highest SOD activity with no Pb and lowest SOD activity in 800 mg kg^{-1} Pb with normal irrigation while 50 mg kg^{-1} Pb tended to increase the SOD activity (Fig. 2d). The interaction of Pb and biochar significantly affected the SOD activity (Table 2), but no clear response trends or patterns could be identified (Fig. 2e). The interactive effect of biochar and irrigation on SOD activity was similar to that on soluble sugar concentration (Fig. 2b and f). The effects of Pb POD activity

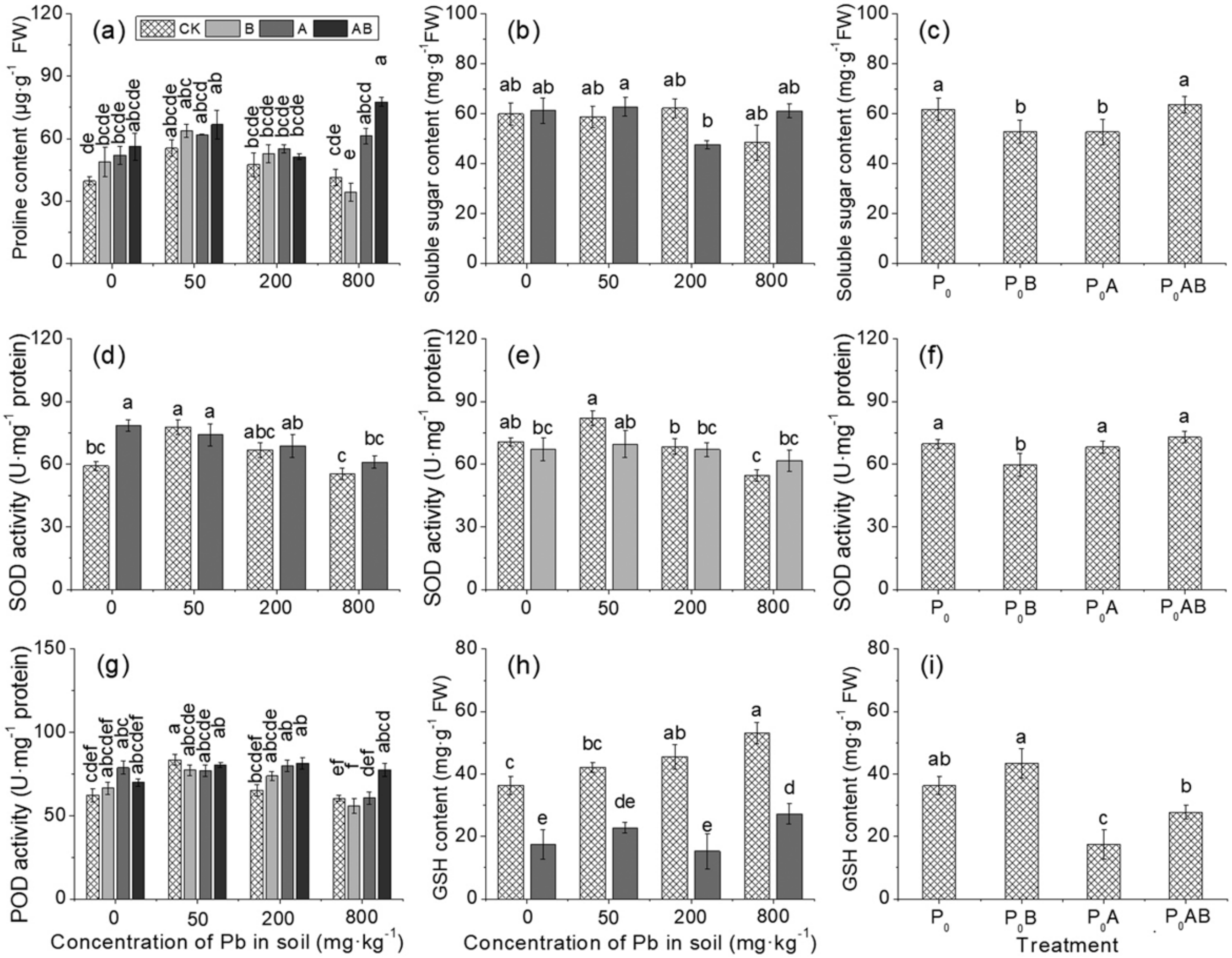

Figure 2: The concentrations of proline (**a**), soluble sugar (**b**, **c**), GSH (**h**, **i**) and the activities of POD (**g**) and SOD (**d**, **e**, **f**) in mulberry seedlings as affected by irrigation method, biochar application and soil Pb concentration. Other explanations are as in Fig. 1. In Figs **c**, **f** and **i**, P0: Pb control treatment, B: biochar application, A: APRI. FW: fresh weight.

varied with irrigation and biochar treatments (Table 2): The highest Pb concentration significantly reduced POD activity except for the combination of biochar application and APRI, while there was no significant difference among other treatment combinations (Fig. 2g). Interestingly, APRI decreased GSH concentration in absence of biochar and the lowest GSH concentration occurred at 200 mg kg^{-1} Pb; GSH concentration showed an increasing trend with increasing of Pb concentration (Fig. 2h). Biochar addition increased GSH concentration under both APRI and regular irrigation but the effect was not statistically significant under regular irrigation. APRI significantly decreased GSH concentration in both biochar treatments (Fig. 2i).

Lead accumulation and translocation

There were significant interactive effects of Pb, biochar application and irrigation method on Pb absorption, translocation and accumulation (Table 2 and Fig. 3a): Leaf PB concentration was significantly higher in the 800 mg kg^{-1} Pb treatment than in other Pb treatments while there was no significant difference between the control and the lowest Pb dosage; Leaf Pb concentrations were substantially higher in the biochar-APRI and 800 mg Pb kg^{-1} treatment combination (39.15 mg Pb kg^{-1} leaf) than in any other treatment combinations (Fig. 3a). Pb concentrations in roots were multiple folds higher than that in leaves and increased with soil Pb concentrations (Fig. 3a and b). Biochar addition significantly increased Pb in roots with a maximum of 828.3 mg·kg^{-1} occurring in the PB_{800} treatment (Fig. 3b). The maximum translocation factor of 0.131 occurred in the treatment combination of APRI, biochar addition and P_{800}, which was substantially larger than all other treatment combinations (Fig. 3c), resulting in much faster increases in leaf Pb with increases in soil and root Pb concentrations than all other treatment combinations (Fig. 4). Biochar addition alone or in combination with APRI generally resulted in the highest bioconcentration factor among the four treatment combinations of biochar and irrigation method in all the Pb treatments (Fig. 3d). The Pb bioconcentration factor showed a decreasing trend with increasing soil Pb concentration under conventional irrigation without biochar addition; however, no clear trends could be identified when biochar was added, or under APRI, or the combination of the two (Fig. 3d).

DISCUSSION

There are three intriguing findings on how the growth traits of mulberry responded to different levels of Pb in the soil and how the responses were modified by the APRI and biochar application. Firstly, mulberry seedlings exhibited high tolerance to soil lead contamination, with no significant decrease in biomass under moderate and severe soil Pb stress. This finding is in agreement with the findings of Zeng *et al.*,

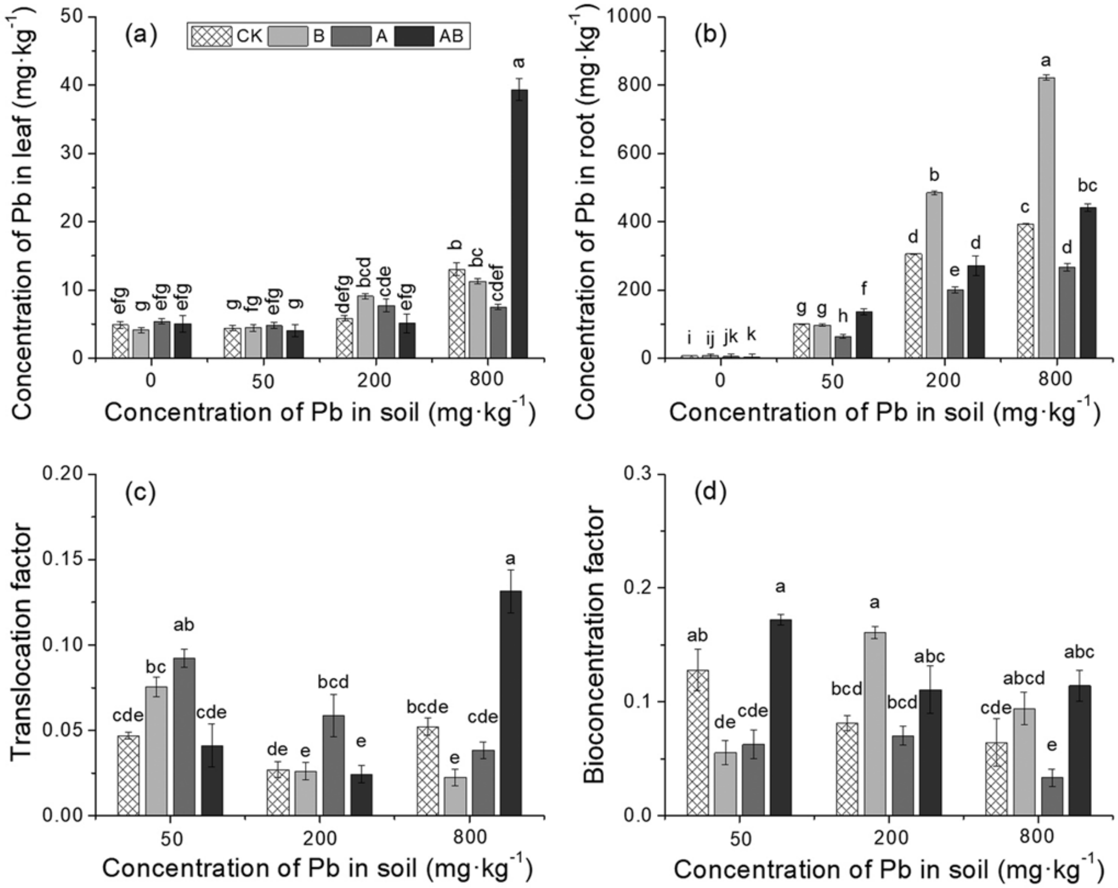

Figure 3: Pb concentrations in leaves and roots (**a**, **b**), translocation factor and bioconcentration factor (**c**, **d**) in mulberry seedlings as affected by APRI and biochar under different Pb concentrations in the soil. Translocation factor = aboveground and belowground Pb content ratio, bioconcentration factor = seedling and substrate Pb concentration ratio. Other explanations are as in Fig. 1.

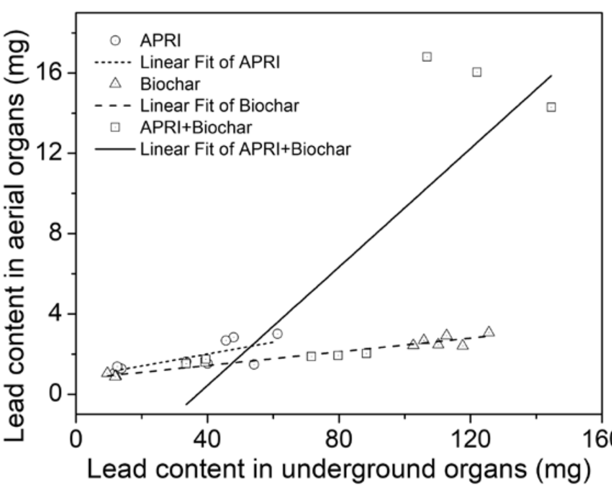

Figure 4: Pb translocation factor under APRI, biochar and their combination. The slopes of the regression lines represent the Pb translocation factor in Pb mulberry seedlings. $y_{\text{APRI}} = 0.03x + 0.819$ ($R^2 = 0.562$); $y_{\text{Biochar}} = 0.017x + 0.756$ ($R^2 = 0.954$); $y_{\text{APRI+Biochar}} = 0.148x - 5.456$ ($R^2 = 0.681$) ($P < 0.05$).

but in contrast to the finding of Qin *et al.* that lead stress inhibited plant growth, particularly in female plants (Qin *et al.* 2018; Zeng *et al.* 2020). Secondly, mild soil Pb stress may impart the apparent hormesis in mulberry seedlings. Under the P_{50} treatment, the biomass increased by 19.4% as compared with the seedlings in the control (i.e. P_0 treatment) and the root–shoot ratio was the highest while the surface area of absorption root was lowest among all the Pb treatments. This result may confirm that Pb-induced hormesis occurred within the threshold concentration range of 25–125 mg Pb kg^{-1} soil, which agrees with the results of a similar study on *Vicia faba* (Wang *et al.* 2010). Hormesis occurs in many plant species in response to heavy metal pollutions; however, there are no consistent conclusions on the functional significance of the response (Morkunas *et al.* 2018). Thirdly, there were apparently some synergistic effects of biochar and APRI on the growth of mulberry seedlings, which confirmed our hypothesis. Although there were interactive effects of Pb, biochar and APRI on biomass and absorption root surface area, the synergistic effect of biochar and APRI on those parameters were generally consistent among different Pb concentrations (Fig. 1a and d). In the current study, APRI alone slightly promoted the growth of mulberry seedlings; APRI in combination with biochar application, however, resulted in a remarkable enhancement in growth and stress resistance. Furthermore, under the condition of biochar application and APRI, there was no significant difference in seedling biomass among different soil Pb concentrations, suggesting that the combination of biochar and APRI may have increased the Pb resistance of mulberry seedlings. It is generally believed that exogenous biochar can exert a profound influence on plant roots through improving the physicochemical environment in the soil, nutrient availability and microbial activities (Jianhong *et al.* 2020; Tu *et al.* 2020). There may be various physiological and/or biochemical traits responsible for the positive effects of exogenous biochar or APRI on phytoremediation.

Highly biotoxic lead ions can destroy the biological activity of macromolecules and plasma membrane structures, resulting in the loss of biological functions, cell electrolyte leakage and loss of turgor (Peng *et al.* 2020a; Zhang *et al.* 2020). Therefore, it is crucial for plants to have the capacity of effective osmotic regulation and the ability to maintain reasonable water relations for them to survive and grow under lead stress. In this study, foliar proline content in mulberry seedlings grown under APRI and biochar was significantly higher under severe soil lead stress than those without biochar and APRI. However, there was no significant difference in soluble sugar content, suggesting that proline may have played a critical role in maintaining the growth of mulberry seedlings under the severe lead stress. This conclusion is consistent with the finding of other studies that increases in proline can improve plant resistance to abiotic stress (Kumar and Prasad 2018; Ramanjulu and Sudhakar 2000). As a small amino acid with the highest solubility, proline may play a critical role in the plant's acclimation to heavy metal pollution in the soil.

Heavy metal ions in plants not only upset osmotic regulation but also cause oxidative stress and damages to physiological processes and metabolisms (Zhang *et al.* 2020). It is believed that plants maintain a rational redox environment by quenching excess free radicals through enzymatic and nonenzymatic mechanisms (Morkunas *et al.* 2018). The results of this study show that SOD activities in mulberry seedlings declined with increasing Pb concentration in the soil, but the impact varied with irrigation method and biochar treatment. Furthermore, biochar and APRI significantly increased POD activities under severe Pb stress (P_{800}). Thus, it may be inferred that biochar and APRI induced POD to cope with oxidative stress caused by the high soil Pb concentration (Alves *et al.* 2018).

Nonenzymatic compounds, such as GSH, can counteract the oxidative stress actions of heavy metals in plants (Barrameda-Medina *et al.* 2014). GSH not only serves as an antioxidant through the ascorbic–GSH cycle but also as an important clathrate in plants, which can contain heavy metal ions and thus reduce their toxicity (Hasanuzzaman *et al.* 2017). It is somewhat surprising that APRI in this study remarkably reduced the GSH content, but increased NPT content, and that biochar did not have a significant effect on GSH content. The results indicate that the nonenzymatic mechanisms in mulberry were related to the degree of soil lead stress and APRI. These results are consistent with the findings of some other studies and suggest that GSH was involved in the detoxification of ROS and the chelation of heavy metal ions (Barrameda-Medina *et al.* 2014; Cheng *et al.* 2015).

Soil resident lead can have a significant impact on plant roots, particularly absorbing roots (Qin *et al.* 2018). However, in this study, the lead treatments did not significantly reduce the root–shoot ratio. It is possible that the seedlings increased their root-regenerating capacity in response to the lead treatments as found in other plant species exposed to heavy metal ions in the soil (Huskey *et al.* 2018). APRI appeared to have promoted root regeneration, improved the root–shoot ratio and enhanced resistance to soil lead stress. We found that roots were the primary organ for Pb accumulation in mulberry and the accumulation increased with increasing Pb concentration in the soil, which are consistent with the findings of Xinxin *et al.* (2020). The application of biochar significantly increased Pb absorption in the root in seedlings subjected to moderate and severe lead stress. The reason for this response pattern is not clear, but it may be associated with the ability of biochar to change the bioavailability of lead and/or soil pH (Jianhong *et al.* 2020; Tu *et al.* 2020). However, APRI substantially reduced the concentration of lead ions in the root system. There were two possible explanations for this resulter4. Firstly, the alternating dry–wet cycles of APRI reduced the total absorption of all ions from the soil and/or improved the ability of the root system to select against harmful ions in nutrient absorption (Consoli *et al.* 2017). Secondly, APRI accelerated the rate of mortality and regeneration of absorption roots and some of the lead ions were locked in dead roots.

In conclusion, this study demonstrated that the mulberry had a strong ability to acclimate to soil lead contamination and that the combination of biochar and APRI had synergistic effects on improving the ability of mulberry seedlings to resist and sequester lead in the soil. The results suggest that growing mulberry in combination with the application of biochar and APRI may represent a viable option to remediate and reclaim the productivity of lead-contaminated soils.

Funding

This study was funded by the Innovative Foundation of Mulberry and Silkworm Research Institute, Chinese Academy of Agricultural Sciences (16JK005).

Acknowledgements

We are thanks Dr Jialing Cheng for his help with greenhouse work and thanks Mr Yongqing Lin for providing seedlings. We are grateful to the two anonymous reviewers for their helpful comments.

Conflict of interest statement. The authors declare that they have no conflict of interest.

REFERENCES

Alves RC, de Medeiros AS, Nicolau MCM, *et al.* (2018) The partial root-zone saline irrigation system and antioxidant responses in tomato plants. *Plant Physiol Biochem* **127**:366–79.

Asgari Lajayer B, Ghorbanpour M, Nikabadi S (2017) Heavy metals in contaminated environment: destiny of secondary metabolite biosynthesis, oxidative status and phytoextraction in medicinal plants. *Ecotoxicol Environ Saf* **145**:377–90.

Barrameda-Medina Y, Montesinos-Pereira D, Romero L, *et al.* (2014) Role of GSH homeostasis under Zn toxicity in plants with different Zn tolerance. *Plant Sci* **227**:110–21.

Berni R, Luyckx M, Xu X, *et al.* (2019) Reactive oxygen species and heavy metal stress in plants: impact on the cell wall and secondary metabolism. *Environ Exp Bot* **161**:98–106.

Cheng MC, Ko K, Chang WL, *et al.* (2015) Increased glutathione contributes to stress tolerance and global translational changes in *Arabidopsis*. *Plant J* **83**:926–39.

Consoli S, Stagno F, Vanella D, *et al.* (2017) Partial root-zone drying irrigation in orange orchards: effects on water use and crop production characteristics. *Eur J Agron* **82**:190–202.

Dodd IC, Puértolas J, Huber K, *et al.* (2015) The importance of soil drying and re-wetting in crop phytohormonal and nutritional responses to deficit irrigation. *J Exp Bot* **66**:2239–52.

Ennahli S, Kadir S, Khatamian H, *et al.* (2015) Physiological and biochemical responses to partial root-zone drying of three grape cultivars. *Theor Exp Plant Physiol* **27**:141–56.

Gerhardt KE, Gerwing PD, Greenberg BM (2017) Opinion: Taking phytoremediation from proven technology to accepted practice. *Plant Sci* **256**:170–85.

Hasanuzzaman M, Nahar K, Anee TI, *et al.* (2017) Glutathione in plants: biosynthesis and physiological role in environmental stress tolerance. *Physiol Mol Biol Plants* **23**:249–68.

He N, Zhang C, Qi X, *et al.* (2013) Draft genome sequence of the mulberry tree *Morus notabilis*. *Nat Commun* **4**:2445.

Huskey DA, Curlango-Rivera G, Root RA, *et al.* (2018) Trapping of lead (Pb) by corn and pea root border cells. *Plant Soil* **430**:205–17.

Jianhong L, ShanLi W, Jingmin Z, *et al.* (2020) Coconut-fiber biochar reduced the bioavailability of lead but increased its translocation rate in rice plants: elucidation of immobilization mechanisms and significance of iron plaque barrier on roots using spectroscopic techniques. *J Hazard Mater* **389**:122117.

Kavitha B, Reddy PVL, Kim B, *et al.* (2018) Benefits and limitations of biochar amendment in agricultural soils: a review. *J Environ Manage* **227**:146–54.

Kumar A, Prasad MNV (2018) Plant-lead interactions: transport, toxicity, tolerance, and detoxification mechanisms. *Ecotoxicol Environ Saf* **166**:401–18.

Liu Y, Willison JH, Wan P, *et al.* (2016) Mulberry trees conserved soil and protected water quality in the riparian zone of the Three Gorges Reservoir, China. *Environ Sci Pollut Res Int* **23**:5288–95.

Livne-Luzon S, Shemesh H, Gilor O, *et al*. (2016) The effect of temporal variation in soil carbon inputs on interspecific plant competition. *J Plant Ecol* **9**:564–75.

Mahdavian K, Ghaderian SM, Schat, H (2016) Pb accumulation, Pb tolerance, antioxidants, thiols, and organic acids in metallicolous and non-metallicolous *Peganum harmala* L. under Pb exposure. *Environ Exp Bot* **126**:21–31.

Ming L, Yuqi P, Congying C, *et al*. (2019) Application of economic plant for remediation of cadmium contaminated soils: three mulberry (*Moms alba* L.) varieties cultivated in two polluted fields. *Chemosphere* **236**:124379.

Morkunas I, Woźniak A, van Mai C, *et al*. (2018) The role of heavy metals in plant response to biotic stress. *Molecules* **23**:2320.

Peng S, Wu L, Seyler BC, *et al*. (2020a) The combined effects of Cu and Pb on the sex-specific growth and physiology of the dioecious *Populus yunnanensis*. *Environ Res* **184**:109276.

Peng Z, Zhaohui G, Xiyuan X, *et al*. (2020b) Physiological stress responses, mineral element uptake and phytoremediation potential of *Morus alba* L. in cadmium-contaminated soil. *Ecotoxicol Environ Saf* **189**:109973.

PRC Ministry of the Environment and the Ministry of Natural Resources (2014) *Investigation Report on the State of Soil Pollution in the Republic of China*. http://www.gov.cn/foot/2014-04/17/content_2661768.htm

Qin F, Liu G, Huang G, *et al*. (2018) Zinc application alleviates the adverse effects of lead stress more in female *Morus alba* than in males. *Environ Exp Bot* **146**:68–76.

R Development Core Team (2019) R: a language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing.

Ramanjulu S, Sudhakar C (2000) Proline metabolism during dehydration in two mulberry genotypes with contrasting drought tolerance. *J Plant Physiol* **157**:81–5.

Sánchez-Salcedo EM, Mena P, García-Viguera C, *et al*. (2015) (Poly)phenolic compounds and antioxidant activity of white (*Morus alba*) and black (*Morus nigra*) mulberry leaves: their potential for new products rich in phytochemicals. *J Funct Foods* **18**:1039–46.

Sun L, Zhang C, Li J, *et al*. (2016) Assessing the sustainability of lead utilization in China. *J Environ Manage* **183**:275–9.

Tu C, Wei J, Guan F, *et al*. (2020) Biochar and bacteria inoculated biochar enhanced Cd and Cu immobilization and enzymatic activity in a polluted soil. *Environ Int* **137**:105576.

Ullah A, Heng S, Munis MFH, *et al*. (2015) Phytoremediation of heavy metals assisted by plant growth promoting (PGP) bacteria: a review. *Environ Exp Bot* **117**:28–40.

Wan X, Lei M, Chen T, *et al*. (2017) Safe utilization of heavy-metal-contaminated farmland by mulberry tree cultivation and silk production. *Sci Total Environ* **599–600**:1867–73.

Wang L, Shi H, Wu J, *et al*. (2016) Alternative partial root-zone irrigation enhances leaf flavonoid accumulation and water use efficiency of *Ginkgo biloba*. *New Forests* **47**:377–91.

Wang CR, Tian Y, Wang XR, *et al*. (2010) Hormesis effects and implicative application in assessment of lead-contaminated soils in roots of *Vicia faba* seedlings. *Chemosphere* **80**:965–71.

Wang M, Zhu Y, Cheng L, *et al*. (2018) Review on utilization of biochar for metal-contaminated soil and sediment remediation. *J Environ Sci (China)* **63**:156–73.

Xinxin L, Xiang L, Wei L, *et al*. (2020) Toxicity, migration and transformation characteristics of lead in soil-plant system: effect of lead species. *J Hazard Mater* **395**:122676.

Yang Q, Li Z, Lu X, *et al*. (2018) A review of soil heavy metal pollution from industrial and agricultural regions in China: pollution and risk assessment. *Sci Total Environ* **642**:690–700.

Zeng P, Huang F, Guo Z, *et al*. (2020) Physiological responses of *Morus alba* L. in heavy metal(loid)-contaminated soil and its associated improvement of the microbial diversity. *Environ Sci Pollut Res Int* **27**:4294–308.

Zhang H, Li X, Xu Z, *et al*. (2020) Toxic effects of heavy metals Pb and Cd on mulberry (*Morus alba* L.) seedling leaves: photosynthetic function and reactive oxygen species (ROS) metabolism responses. *Ecotoxicol Environ Saf* **195**:110469.