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Abscisic acid improves the phytoremediation ability of the aquatic accumulator plant Nasturtium officinale R. Br. in cadmium-contaminated soil

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Abstract

Previous studies report significant contamination of farmland soil by cadmium (Cd). Cadmium contamination increases the risk of diseases after consumption of grain crops with high Cd levels. Hyperaccumulators or accumulators have been widely used to bioremediate Cd-contaminated farmland soil. Studies report that aquatic hyperaccumulators or accumulators are rarely used in phytoremediation, or their phytoremediation efficiencies are low. A pot experiment was carried out to explore the effects of abscisic acid (ABA) on the cadmium (Cd) accumulation (phytoremediation) of emerged aquatic accumulator plant *Nasturtium officinale* R. Br. ABA application increased the shoot biomass, peroxidase (POD) activity, superoxide dismutase (SOD) activity, catalase (CAT) activity, and soluble protein content of *N. officinale* under Cd-contamination condition. The application of ABA at 5, 10, and 20 μmol/L increased the chlorophyll and carotenoid contents in *N. officinale*. The application of ABA also increased the shoot Cd content and shoot Cd extraction of *N. officinale*. The application of ABA at 1, 5, 10, and 20 μmol/L increased the shoot Cd extraction by 11.65, 25.27, 37.91, and 58.52%, respectively, compared with the control. In addition, ABA concentration had a linear regression relationship with the root biomass, shoot biomass, root Cd extraction, and shoot Cd extraction. Correlation, grey relational, and path analyses showed that the root biomass, shoot biomass, root Cd extraction, root Cd content, POD activity, SOD activity, and soluble protein content were significantly associated with the shoot Cd extraction. These findings indicate that ABA application can improve the phytoremediation ability of *N. officinale* under Cd-contaminated flooded fields.

Introduction

Environmental pollution from heavy metal has significantly increased due to the increase in industrialization [1]. Cadmium (Cd) is a toxic heavy metal and is the highest source of heavy metal contamination on farmlands [2]. The area of Cd-contaminated paddy soil increases yearly [3, 4]. Cadmium has high mobility and is easily absorbed and accumulated by crops, thus poses a high risk to human health when carried through the food chain [4]. High levels of Cd in the soil significantly affect the growth and development of crops [5, 6]. Under Cd stress, the chloroplast ultrastructure of plants is destroyed, and the net photosynthetic rate, leaf transpiration, and chlorophyll synthesis are inhibited, which results in the reduced photosynthesis of plants [7]. Meanwhile, more reactive oxygen species (ROS) are produced in plants, which lead to the oxidative damage to plants [8, 9]. Therefore, effective measures should be formulated for remediation of the Cd-contaminated soil and reduction of Cd uptake in crops.

The remediation methods of Cd-contaminated soil are mainly grouped into three categories: physical remediation, chemical remediation, and bioremediation [10]. Phytoremediation method, one of the bioremediation approaches, has several advantages, such as low cost of implementation and lack of secondary pollution compared with the other remediation methods [11]. Phytoremediation mainly involves the use of plants that are known as hyperaccumulator and accumulator plants. However, most of these plants have several shortcomings, including slow growth, low biomass, and a long remediation cycle, which limit their application in phytoremediation [12, 13]. Therefore, researchers should explore approaches for improving the phytoremediation abilities of known hyperaccumulator or accumulator plants to the Cd-contaminated soil. Agronomic measures currently used to enhance the phytoremediation abilities of hyperaccumulator or accumulator plants, including the intercropping, grafting, disposing of straws to the fields, and application of plant hormones [14–19]. Plant hormones, mainly include auxin, gibberellin, cytokinin, ethylene, abscisic acid (ABA), brassinosteroids, jasmonate, salicylic acid, and strigolactones, are compounds that affect plant growth by regulating plant physiological metabolism or increasing plant resistant to stress [18–20]. ABA is an important signaling substance, which plays an essential role in plant resistance to drought, salt damage, waterlogging, chilling damage, as well as diseases, and is known as the "stress hormone" [21]. The ABA content in plants rapidly increases to enhance plant resistance when plants exposed to stress conditions [22, 23]. When the exogenous ABA application, the endogenous ABA concentration accumulates in plants [24, 25], which drives the stomatal closure and regulates the expressions of osmoregulation and antioxidant enzyme related genes, thereby reducing the transpiration rate and increasing the osmoregulatory substances contents and antioxidant enzyme activity [26]. Application of ABA improved the Cd tolerance in wheat and barley seedlings [27, 28] and decreased the Cd content in shoots of non-heading cabbage and rice seedlings [29, 30]. In addition, ABA application improved the tolerance of tomatoes and poplars to Cd stress by increasing the activities of their antioxidant enzymes and photosynthetic pigments contents [31, 32]. Application of ABA promoted the growth of *Cyphomandra beracea* Sendt. and lettuce, increased the contents of photosynthetic pigments, and decreased Cd contents in these plants under Cd stress [33, 34]. For Cd-accumulator or Cd-hyperaccumulator plants, ABA treatment increased the biomass, antioxidant enzyme activity, soluble protein content, soluble sugar content, and Cd content of Cd-accumulator plant *Stellaria media* (L.) Cyr., thereby improving its phytoremediation capability [35]. Different concentrations of ABA (1–40 μmol/L) increased the Cd contents in two ecotypes of the Cd-hyperaccumulator *Bidens pilosa* L. but decreased the biomass and photosynthetic pigments contents. Application of 40 μmol/L ABA increased the Cd extraction efficiencies (phytoremediation capabilities) of these plants, whereas other concentrations of ABA decreased Cd extraction efficiencies [36]. The different concentrations of ABA (1–40 μmol/L) promoted the photosynthetic pigments synthesis and the growth of two ecotypes of the Cd-hyperaccumulator *Solanum photeinocarpum* Nakamura et S. Odashima, and also improved the phytoremediation capabilities of the varieties for Cd [37]. These results show that ABA has different effects on the Cd uptake and growth of common, hyperaccumulator, and accumulator plants, which may be related to the different physiological responses of these plant types to Cd. These findings also indicate that ABA can be used to improve the phytoremediation capabilities of hyperaccumulator and accumulator plants.

Nasturtium officinale R. Br. is an emerged aquatic plant and a member of the Cruciferae family. *N. officinale* is a Cd-accumulator plant with various advantages such as ease of propagation (seed or cuttings), fast growth, and wide distribution [38, 39]. These features imply that *N. officinale* can be used for the phytoremediation of Cd-contaminated flooded soil. However, the low phytoremediation efficiency of *N. officinale* should be improved for its application in the field. It was hypothesized that if exogenous ABA is applied on *N. officinale*, its Cd-phytoremediation capacity could be improved. Therefore, we planted *N. officinale* in Cd-contaminated soil, and ABA was applied to the leaves of *N. officinale* to to improve the phytoremediation efficiency of *N. officinale*. This study sought to explore whether applying ABA on *N. officinale* could improve its phytoremediation efficiency. The findings obtained will provide information on phytoremediation of Cd-contaminated flooded fields.

Materials and methods

Materials

The seedlings of *N. officinale* were collected from a trench at the farmland of Ya'an Campus of Sichuan Agricultural University (29° 59' N, 102° 59' E). Cuttings of about 10 cm in length were obtained from the shoot tips.

Soil samples were collected from the fields around the Chengdu Campus of Sichuan Agricultural University (30°42'N, 103°51'E). The soil samples were analyzed to ensure they had no detectable Cd levels. The soil samples were pre-processed as described by Lin *et al.* (2020) [40] for air-drying and crushing, and the soil physicochemical properties (Table 1) were the same as the report of Tang *et al.* (2022) [41].

Table 1
The physicochemical properties of soil [41]

Soil type	pH value	Total N content (g/kg)	Total P content (g/kg)	Total K content (g/kg)	Alkali hydrolysis N content (mg/kg)	Available P content (mg/kg)	Available K content (mg/kg)	Total Cd content (mg/kg)
Fluvo-aquic	7.09	1.50	0.76	18.02	94.82	63.30	149.59	Not detected

ABA (analytical grade) was purchased from the Beijing Solarbio Science & Technology Co., Ltd.

Experimental design

The soil samples (3.0 kg) were transferred into a 25 cm × 10 cm (diameter × height) plastic pot in August 2020 and treated with the Cd (in the form of $CdCl_2 \cdot 2.5H_2O$), and watered to maintain the water depth at 2–3 cm above the soil surface [41]. The final total Cd concentration in soil was 5 mg/kg according to Tang *et al.* (2022) [41]. After that, the pots were placed in a canopy, and the pot soil was flooded for a month. Three uniform cuttings of *N. officinale* were planted into each pot in September 2020 at a 5 cm depth into the Cd-treated soil. The pots were watered to maintain the water depth at 2–3 cm above the soil surface during the first week. The water depth was adjusted to 5 cm above the soil surface after the first week. Then, the different concentrations (0, 1, 5, 10 and 20 μmol/L) of ABA were sprayed on the leaves (both sides) of plant seedlings until the solution started dripping [42]. Each pot was sprayed 25 mL ABA solution. Different concentrations of ABA were re-sprayed again a week later of first spraying to strengthen the role of ABA [36]. Each treatment was conducted in triplicates (three pots). The pots were watered every day to keep the water depth at 5 cm above the soil surface.

Determination of parameters

One month after the first ABA treatment, the fourth fully expanded leaf of each main stem was collected to determine the contents of photosynthetic pigments (total chlorophylls and carotenoids). In addition, the fifth fully expanded leaf of each main stem was harvested for determination of activities of antioxidant enzymes [peroxidase (POD), superoxide dismutase (SOD), and catalase (CAT)] and soluble protein content. To determine the chlorophyll and carotenoid contents, the fresh leaf samples were cut into pieces and were soaked in the ethanol and acetone (1:1, v/v) mixture solution in dark for 24 h [43], and then the extraction solution was used to determine the chlorophyll and carotenoid contents using a spectrophotometer (Summit, Shanghai, China) [44]. Also, the fresh leaf samples were homogenized in potassium phosphate extraction buffer, centrifuged, and the supernatant was used for determining the SOD activity, POD activity, CAT activity, and soluble protein content using the nitroblue tetrazolium photoreduction method, guaiacol colorimetric method, ultraviolet absorption method, and Coomassie brilliant blue method, respectively [40, 44].

After the determination of physiological parameters, the whole plants were harvested and washed. The plants were separated to roots and shoots, and dried in an oven (80°C). The dry weights (biomass) of roots and shoots were weighed using an electronic balance. The dried plants were then ground to a fine powder and digested in the nitrate acid and perchloric acid (4:1, v/v) solution at 200°C [40]. The digested solution was used to determine the root Cd and shoot Cd contents using an ICAP6300 ICP spectrometer (Thermo Scientific, Waltham, MA, USA). The Cd measurements were checked with certified standard reference material (GBW-07602; bush branches and leaves) obtained from the China National Center for Standard Reference Materials [45]. The Cd extraction was calculated as plant Cd content × plant biomass [46]. After the pot water evaporated, soil samples in the experimental pots were collected, air-dried, and sieved. Subsequently, the soil pH value was evaluated using a pH meter, and the soil exchangeable Cd concentration was determined using an ICAP6300 ICP spectrometer, respectively after extraction with DTPA-TEA [40, 47].

Statistical analysis

Statistical analysis was carried out using SPSS 20.0.0 software (IBM, Chicago, IL, USA). The one-way analysis of variance and Duncan's Multiple Range Test ($P < 0.05$) were used to analyze the differences among treatments. The relationship between ABA concentration and the root biomass, shoot biomass, root Cd extraction, or shoot Cd extraction was evaluated through regression analysis, and the linear regression relationship was selected by curve fitting. Pearson's correlation analysis was conducted to determine the relationships among the different indicators. The grey relational analysis method was used to evaluate the relationships between the different indicators with the shoot Cd extraction [48, 49]. The path analysis method was used to evaluate the causal relationships among the different indicators with the shoot Cd extraction [49].

Results

Biomass

The root biomass and shoot biomass of *N. officinale* increased with the ABA concentration going up (Fig. 1A, 1B). The root biomass was increased by 21.80 and 28.57% after application of 10 and 20 μmol/L ABA compared with the control, respectively, whereas no significant effect was observed after treatment with 1 and 5 μmol/L ABA. The shoot biomass was higher after treatment with ABA compared with the control. The shoot biomass increased by 9.76, 15.75, 25.51, and 41.95% after the treatment of 1, 5, 10, or 20 μmol/L ABA, respectively, compared with the control. In addition, ABA concentration (x) had a linear regression relationship with the root biomass (y) ($y = 0.002x + 0.134$, $R^2 = 0.868$, $P = 0.000$) and shoot biomass (y) ($y = 0.011x + 0.612$, $R^2 = 0.947$, $P = 0.000$).

Photosynthetic pigment content

The contents of chlorophyll and carotenoid in leaves of *N. officinale* were higher after the treatments of 5, 10, and 20 μmol/L ABA compared with the control (Fig. 2A). However, no significant effects were observed after spraying with 1 μmol/L ABA (Fig. 2A, 2B). The total chlorophyll content increased by 3.40, 6.12, and 7.48%, and the carotenoid content increased by 5.77, 5.77, and 7.69% after spraying with 5, 10, or 20 μmol/L ABA, respectively, relative to the control.

Antioxidant enzyme activity and soluble protein content

The application of ABA increased the POD, SOD, and CAT activities of *N. officinale* leaves (Fig. 3A, 3B, 3C). The POD activity increased by 5.40, 10.97, 14.97, and 21.67% respectively, whereas the SOD activity increased by 3.91, 11.16, 23.34, and 35.19%, after application of 1, 5, 10, or 20 μmol/L ABA, respectively, relative to the respective control. Compared with the control, the CAT activity increased by 20.15, 67.84, 140.60, and 204.93% after the treatment with 1, 5, 10, or 20 μmol/L ABA, respectively. The application of ABA also increased the soluble protein content in *N. officinale* leaves (Fig. 3D). Compared with the control, the soluble protein content increased by 13.09, 30.63, 49.01, and 90.47% after application of 1, 5, 10, or 20 μmol/L ABA, respectively.

Cd content

The application of ABA increased the contents of root Cd and shoot Cd in *N. officinale* (Fig. 4A, 4B). Compared with the control, the application of ABA at 10 and 20 μmol/L increased the root Cd content by 9.29 and 13.45%, respectively, whereas the application of ABA at 1 and 5 μmol/L had no significant effects (Fig. 4A). Compared with the control, the application of ABA at 5, 10, and 20 μmol/L increased the shoot Cd content by 8.22, 9.86, and 11.67%, respectively, whereas the application of ABA at 1 μmol/L ABA had no significant effect (Fig. 4B).

Cd extraction

The application of ABA increased the extractions of root Cd and shoot Cd in *N. officinale* (Fig. 5A, 5B). Compared with the control, the application of ABA at 10 and 20 μmol/L increased the root Cd extraction by 33.15 and 45.90%, respectively, whereas the application of ABA at 1 and 5 μmol/L had no significant effects. Compared with the control, the application of ABA at 1, 5, 10, and 20 μmol/L increased the shoot Cd extraction by 11.65, 25.27, 37.91, and 58.52%, respectively. Moreover, ABA concentration (x) had a linear regression relationship with the root Cd extraction (y) ($y = 0.081x + 3.519$, $R^2 = 0.868$, $P = 0.000$) and shoot Cd extraction (y) ($y = 0.554x + 21.746$, $R^2 = 0.927$, $P = 0.000$).

Soil pH value and soil exchangeable Cd concentration

The application of ABA at 10 and 20 μmol/L decreased the soil pH value compared with the control, whereas the application of ABA at 1 and 5 μmol/L had no significant effects (Fig. 6A). Treatment with 20 μmol/L ABA increased the soil exchangeable Cd concentration compared with the control, whereas the application of ABA at 1, 5, and 10 μmol/L had no significant effects (Fig. 6B).

Correlation analysis

The root biomass and shoot biomass were highly significantly ($P < 0.01$) positively correlated with the chlorophyll content, carotenoid content, POD activity, SOD activity, CAT activity, and soluble protein content (Table 2). The root Cd content, shoot Cd content, root Cd extraction, and shoot Cd extraction were highly significantly ($P < 0.01$) or significantly ($P < 0.05$) positively correlated with the root biomass, shoot biomass, chlorophyll content, carotenoid content, POD activity, SOD activity, CAT activity, soluble protein content, and soil exchangeable Cd concentration, and highly significantly ($P < 0.01$) negatively correlated with the soil pH value. The root Cd content and shoot Cd content were highly significantly ($P < 0.01$) positively correlated with the root Cd extraction and shoot Cd extraction. The shoot Cd extraction was highly significantly ($P < 0.01$) positively correlated with the root Cd extraction. The soil exchangeable Cd concentration was highly significantly ($P < 0.01$) negatively correlated with the soil pH value.

Table 2
Correlations among the different indicators

Indicators	Root biomass	Shoot biomass	Chlorophyll content	Carotenoid content	POD activity	SOD activity	CAT activity	Soluble protein content	Root Cd content	Shoot Cd content	Root Cd extraction	Sh ex
Root biomass	1											
Shoot biomass	0.948**	1										
Chlorophyll content	0.777**	0.842**	1									
Carotenoid content	0.668**	0.707**	0.775**	1								
POD activity	0.919**	0.985**	0.858**	0.766**	1							
SOD activity	0.945**	0.973**	0.887**	0.780**	0.964**	1						
CAT activity	0.961**	0.980**	0.879**	0.762**	0.974**	0.993**	1					
Soluble protein content	0.936**	0.988**	0.828**	0.749**	0.975**	0.980**	0.979**	1				
Root Cd content	0.957**	0.954**	0.758**	0.631*	0.923**	0.899**	0.919**	0.917**	1			
Shoot Cd content	0.872**	0.911**	0.778**	0.802**	0.944**	0.884**	0.910**	0.885**	0.900**	1		
Root Cd extraction	0.996**	0.959**	0.771**	0.660**	0.927**	0.941**	0.957**	0.942**	0.977**	0.884**	1	
Shoot Cd extraction	0.948**	0.995**	0.833**	0.741**	0.990**	0.969**	0.980**	0.982**	0.957**	0.946**	0.960**	1
Soil exchangeable Cd concentration	0.832**	0.897**	0.661**	0.660**	0.852**	0.872**	0.852**	0.921**	0.856**	0.761**	0.855**	0.
Soil pH value	-0.772**	-0.805**	-0.535*	-0.633*	-0.803**	-0.781**	-0.772**	-0.806**	-0.778**	-0.791**	-0.786**	-0

N = 15. **: Correlation is significant at the 0.01 level (2-tailed test). *: Correlation is significant at the 0.05 level (2-tailed test).

Grey relational and path analyses

To further analyze the relationships between the shoot Cd extraction and other indicators, the grey relational analysis was used. The shoot Cd extraction had the grey relational relationships with the other indicators (Fig. 7). The grey correlation coefficients of root biomass, shoot biomass, root Cd extraction, root Cd content, POD activity, SOD activity, and soluble protein content were higher than 0.39. This result indicates that these indicators were closely associated with the shoot Cd extraction with the order of root Cd extraction > shoot biomass > soluble protein content > SOD activity > root biomass > POD activity > root Cd content. In addition, the path analysis showed that the shoot biomass and root Cd extraction had the highest positive direct effects on the shoot Cd extraction, and the root biomass had the highest negative direct effect on the shoot Cd extraction. The root biomass, POD activity, SOD activity, soluble protein content, and root Cd content had the highest positive indirect effects on the shoot Cd extraction (Table 3).

Table 3
Path coefficients of the different indicators with the shoot Cd extraction.

Factor	Direct effect	Indirect effect												
		Total	X1→Y	X2→Y	X3→Y	X4→Y	X5→Y	X6→Y	X7→Y	X8→Y	X9→Y	X10→Y	X11→Y	X1
X1	-0.8265	1.7746		0.5199	-0.0058	-0.0004	0.0434	-0.0398	0.1708	0.0271	-0.212	0.1914	1.0802	-0.(
X2	0.5487	0.4467	-0.7832		-0.0063	-0.0004	0.0465	-0.0409	0.1742	0.0287	-0.2113	0.2	1.0396	-0.(
X3	-0.0074	0.8409	-0.6422	0.4619		-0.0005	0.0405	-0.0373	0.1562	0.024	-0.168	0.1709	0.8355	-0.(
X4	-0.0006	0.7419	-0.5518	0.3879	-0.0058		0.0362	-0.0328	0.1354	0.0217	-0.1399	0.1761	0.715	-0.(
X5	0.0472	0.9429	-0.7595	0.5405	-0.0064	-0.0005		-0.0406	0.173	0.0283	-0.2045	0.2074	1.0054	-0.(
X6	-0.0421	1.0108	-0.7808	0.5339	-0.0066	-0.0005	0.0455		0.1765	0.0284	-0.1992	0.1942	1.0196	-0.(
X7	0.1777	0.8026	-0.7944	0.5378	-0.0065	-0.0005	0.0459	-0.0418		0.0284	-0.2037	0.1998	1.0378	-0.(
X8	0.029	0.9532	-0.7732	0.5422	-0.0062	-0.0004	0.046	-0.0412	0.1741		-0.2031	0.1944	1.0208	-0.(
X9	-0.2215	1.1786	-0.7911	0.5233	-0.0056	-0.0004	0.0436	-0.0378	0.1634	0.0266		0.1976	1.0592	-0.(
X10	0.2196	0.7262	-0.7204	0.4996	-0.0058	-0.0005	0.0446	-0.0372	0.1617	0.0257	-0.1993		0.9579	-0.(
X11	1.0841	-0.1246	-0.8236	0.5261	-0.0057	-0.0004	0.0438	-0.0396	0.1701	0.0273	-0.2165	0.1941		-0.(
X12	-0.0003	0.8848	-0.688	0.4922	-0.0049	-0.0004	0.0402	-0.0367	0.1514	0.0267	-0.1896	0.1672	0.9266	
X13	-0.0001	-0.8178	0.6377	-0.4415	0.004	0.0004	-0.0379	0.0329	-0.1372	-0.0234	0.1724	-0.1737	-0.8518	0.0

Coefficient of determination = 1.0000; Residual path coefficient = 0.0002. X1 = root biomass; X2 = shoot biomass; X3 = chlorophyll content; X4 = carotenoid c
POD activity; X6 = SOD activity; X7 = CAT activity; X8 = soluble protein content; X9 = root Cd content; X10 = shoot Cd content; X11 = root Cd extraction; X12 = s
exchangeable Cd concentration; X13 = soil pH value; Y = shoot Cd extraction.

Discussion

ABA can promote the growth of several plants under Cd stress by increasing their biomass [33, 34]. Under Cd-contaminated condition, ABA increased the biomass of the accumulator plant *S. media* and the Cd-hyperaccumulator plant *S. photeinocarpum* [35, 37]. On the contrary, ABA treatment inhibits the growth of Cd-hyperaccumulator plant *B. pilosa* under Cd-contaminated condition [36]. So, the various effects of ABA on the biomass of different plant species. In the current study, the application of ABA increased the biomass of *N. officinale* under Cd-contaminated conditions, which is consistent with previous studies [33–35, 37]. The ABA concentrations used in this study exhibited a linear regression relationship with the biomass of *N. officinale*. These results indicate that ABA promoted the growth of *N. officinale*, which can be attributed to enhancing resistance to Cd by ABA [21]. In addition, the best ABA concentration for increasing the biomass of *N. officinale* was 20 μmol/L. Whether the greater ABA concentration increases the biomass of *N. officinale* needs further study.

The chloroplast is the main site of plant photosynthesis as well as the main site for ABA synthesis in plant leaves [50]. Studies have shown that spraying plant leaves with ABA increases their contents of chlorophyll and carotenoid and improves their resistance to stress condition [51]. ABA application increases the contents of photosynthetic pigments in several plants under Cd stress or Cd-contaminated conditions [31–34]. Notably, ABA has different effects on the photosynthetic pigments contents of hyperaccumulator or accumulator plants, including increase, decrease, and no effects on the contents [35–37], indicating that the various plant species have different responds to ABA. In this study, ABA increased the contents of chlorophyll and carotenoid in *N. officinale* under Cd-contaminated condition, which is consistent with the results from previous studies [31–34, 37]. This finding indicates that ABA promoted the synthesis of photosynthetic pigment in *N. officinale* under Cd-contaminated condition, which could be related to the ABA receptor ABAR/CHLH in the chloroplast of plant cells, which can catalyze cell chlorophyll synthesis under suitable ABA concentration [52]. On the other hand, ABA is a stress hormone that can be synthesized in plant leaves, and its concentration rapidly increases in plants during stress conditions to enhance plant resistance to stress, hence promoting the synthesis of photosynthetic pigments [21–23, 50].

Cadmium toxicity mainly induces changes in plant physiological metabolism by promoting the production of ROS [8, 9]. ABA is a small signaling molecule that regulates physiological processes, and plays a critical role in Cd stress responses for plants [53]. ABA is involved in the regulation of activities of plant antioxidant enzymes [21]. Application of ABA under Cd stress activates the activities of antioxidant enzymes (SOD, POD, CAT) and increases the content of soluble protein to effectively remove excess ROS in the plants, thus, alleviating the Cd-induced stress to plants [54]. ABA increased the POD and CAT activities and decreased the SOD activity in *C. beracea* under Cd-contaminated condition [34]. Activities of antioxidant enzymes (SOD, POD, and CAT) and soluble protein content of the Cd-accumulator plant *S. media* increased with an increase in ABA concentrations less than 15 mg/L [35]. On the contrary, activities of antioxidant enzymes in *S. media* decreased after treatment with ABA concentrations higher than 15 mg/L under Cd-contaminated condition [35]. In the present study, ABA application increased the activities of antioxidant enzymes (SOD, POD, and CAT) and soluble protein content of *N. officinale* under Cd-contaminated condition, which is consistent with the results reported in previous studies [35, 54]. These results indicate that ABA improved the resistance of *N. officinale* to Cd-contaminated condition. The reason is that ABA is a stress hormone involved in regulating plant antioxidant enzyme activity [21], and ABA can up-regulate the ROS-related genes, increase the glutathione content, and decrease the contents of H_2O_2 and malondialdehyde (MDA) to increase the resistance of plants to stress conditions [55].

Soil pH values can directly affect soil characteristics [56]. A high soil pH value is correlated with low soil Cd bioavailability [57]. In the process of plant growth, the soil pH value is mainly affected by the root secretions of plants, which can increase or reduce the soil pH value of rhizosphere [56, 57]. A previous study reported that applying ABA on *C. beracea* seedlings decreased the soil pH value and reduced the available Cd concentration [34]. However, the application of ABA on hyperaccumulator plant *B. pilosa* increased the soil pH value and available Cd concentration [36]. So, when the various types of plant species are treated by ABA, the different types of root secretions are produced to change the soil pH value and available Cd concentration. In this study, the application of ABA on *N. officinale* decreased the soil pH value and increased the soil exchangeable Cd concentration, which is consistent with the results on hyperaccumulator plants [36], as opposed to common plants [34]. ABA plays a critical role in Cd stress responses for plants, and the production of ABA within plants can be controlled by Cd and other nutrients [53]. The variation in ABA effects can be attributed to the differences in root secretions of the two types of plants [58], which may be related to the different plants respond differently to ABA and needs to be further studied. Applying ABA decreases the Cd uptake of common plants [34] and increases Cd uptake in accumulator or hyperaccumulator plants [35–37]. In this study, ABA application increased the Cd content in *N. officinale*, which is consistent with the studies on other accumulator or hyperaccumulator plants [35–37]. These findings imply that ABA promotes Cd uptake in accumulator or hyperaccumulator plants and reduces Cd uptake in common plants. This effect may be related to enhancing Cd resistance in plants by ABA, ultimately increasing or decreasing the Cd absorption capacity. The mechanism underlying the effect of ABA is through modulation of the physiological metabolism [53]. Changes in metabolism affect the types of root exudates released which may activate or form complexes with soil Cd, ultimately modulating Cd uptakes by plants [21, 58]. The results of this study showed that ABA increased the Cd extraction of *N. officinale*, which is consistent with the results on other accumulator or hyperaccumulator plants [35–37]. This result indicates that ABA improves the phytoremediation ability of *N. officinale*. The concentration of ABA exhibited a linear regression relationship with Cd extraction of *N. officinale*. In addition, the best ABA concentration for increasing the Cd extraction of *N. officinale* was 20 μmol/L. Whether the greater ABA concentration increases the Cd extraction of *N. officinale* needs further study. Moreover, the shoot Cd extraction was significantly correlated with the other indicators. This finding further indicates that ABA modulated physiological and growth indicators to improve the phytoremediation ability of *N. officinale*. Grey relational and path analyses showed that the root biomass, shoot biomass, root Cd extraction, root Cd content, POD activity, SOD activity, and soluble protein content were closely associated with the shoot Cd extraction. This finding implies that these indicators play a significant role in promoting Cd uptake in *N. officinale* under Cd-contaminated conditions. Further studies should be conducted to explore the mechanisms of ABA in modulating the various indicators.

Conclusion

The application of ABA promoted the growth of *N. officinale* under Cd-contaminated condition, and improved the resistance of *N. officinale* to Cd. The application of ABA also promoted the Cd uptake and improved the Cd extraction of *N. officinale*. Root biomass, shoot biomass, root Cd extraction, root Cd content, POD activity, SOD activity, and soluble protein content were closely associated with the shoot Cd extraction. These findings indicate that ABA improved the phytoremediation ability of *N. officinale* under Cd-contaminated conditions. Further studies should be conducted to investigate the mechanism of ABA in promoting Cd accumulation in *N. officinale*.

Declarations

Ethics approval and consent to participate

We all declare that manuscript reporting studies do not involve any human participants, human data, or human tissue. Plant samples were collected from university research area. Study protocol must comply with relevant institutional, national, and international guidelines and legislation. Our experiment follows with the relevant institutional, national, and international guidelines and legislation.

Consent for publication

Not Applicable.

Data availability

All data generated or analyzed during this study are included in this published article.

Competing interests

The authors declare no competing interests.

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Author contributions

X.P., Y.X., Y.Z. and L.L. conceived and designed the research. X.P. and Y.X. wrote the main manuscript text. Y.Z. and L.L. checked and revised the manuscript. X.P., Y.X., W.T., Z.D. and D.L. performed the experiments. X.P. and Y.X. analyzed the data. All authors contributed to this article and approved the submitted version.

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Figures

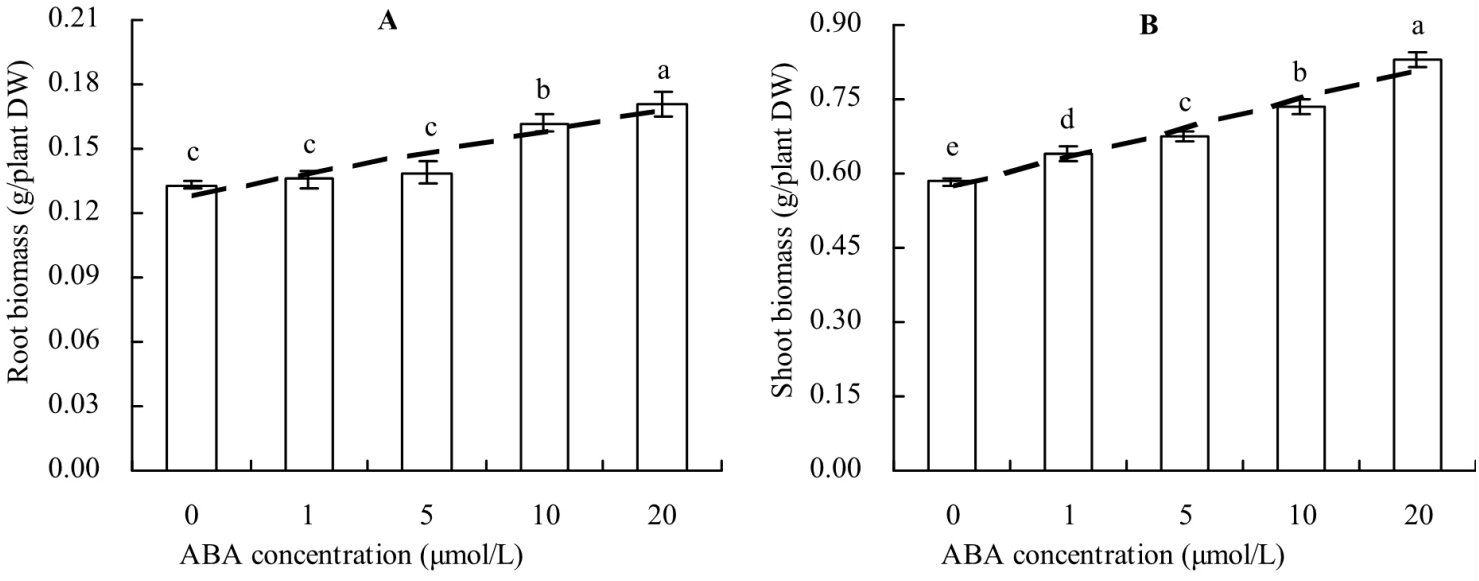


Figure 1

Biomass of *N. officinale*. **A**: root biomass; **B**: shoot biomass. Different lowercase letters indicate significant differences among the treatments (Duncan's Multiple Range Test, $P < 0.05$). DW: dry weight. Dashed line indicates the regression relationship between the ABA concentration and biomass.

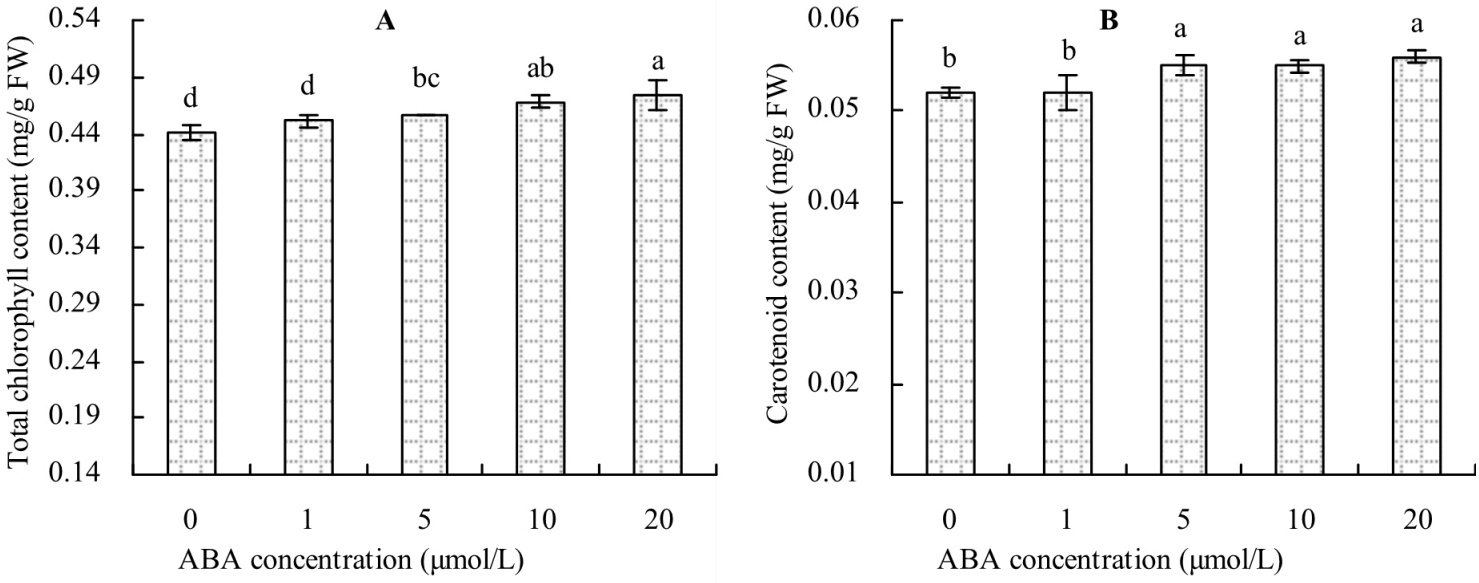


Figure 2

Photosynthetic pigment content in *N. officinale* leaves. **A**: total chlorophyll content; **B**: carotenoid content. Different lowercase letters indicate significant differences among the treatments (Duncan's Multiple Range Test, $P < 0.05$). FW: fresh weight.

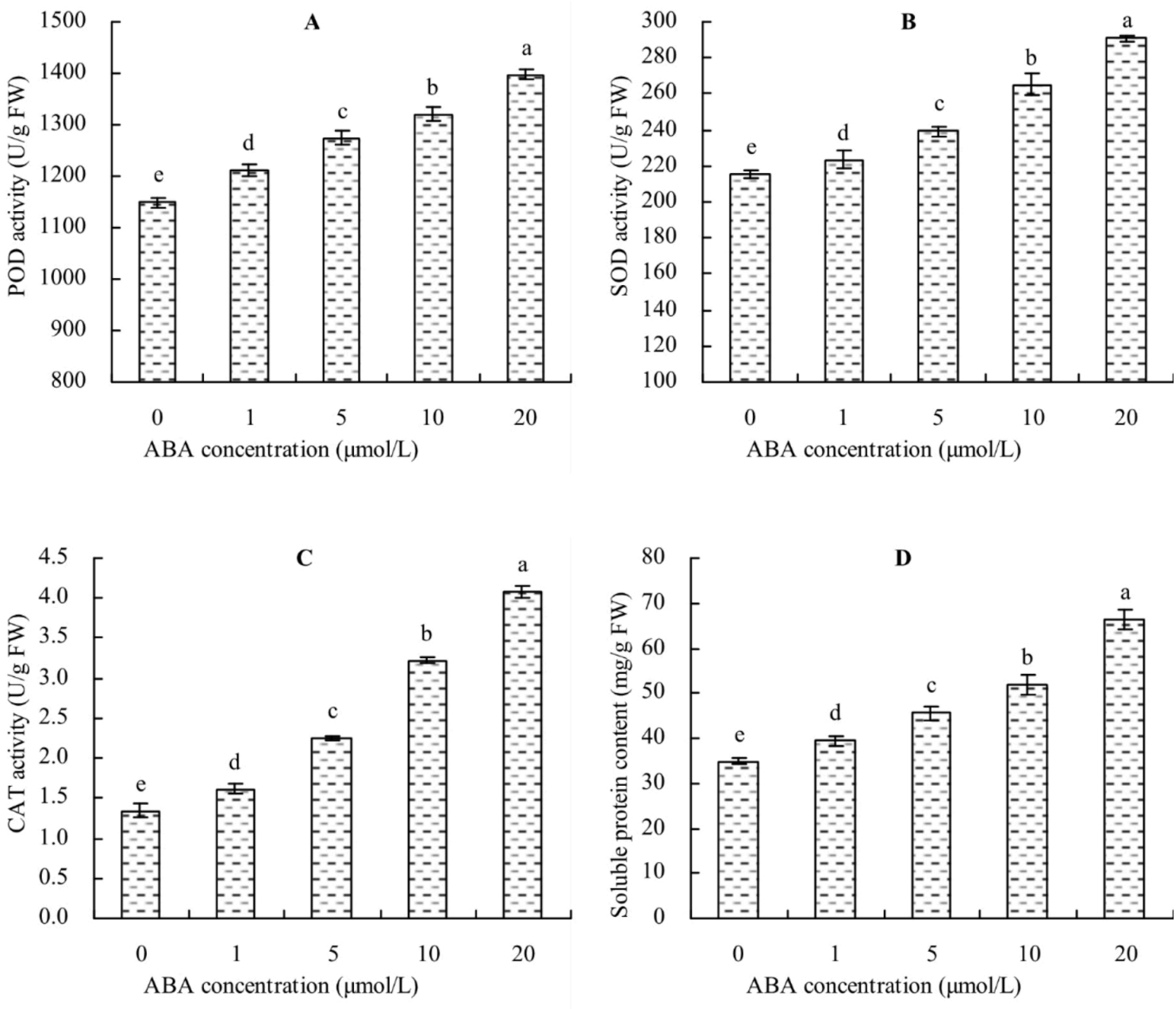


Figure 3

Antioxidant enzyme activity and soluble protein content of *N. officinale*leaves. **A**: POD activity; **B**: SOD activity; **C**: CAT activity; **D**: soluble protein content. Different lowercase letters indicate significant differences among the treatments (Duncan's Multiple Range Test, $P < 0.05$). FW: fresh weight.

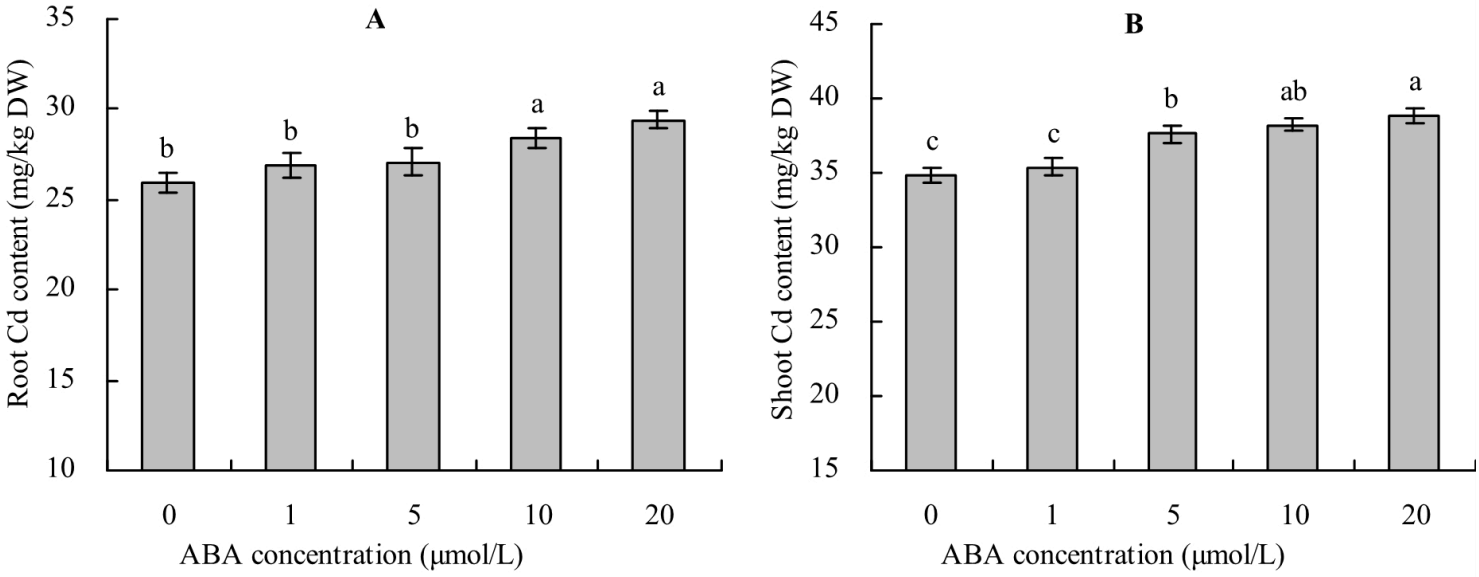


Figure 4

Cd content in *N. officinale*. **A**: root Cd content; **B**: shoot Cd content. Different lowercase letters indicate significant differences among the treatments (Duncan's Multiple Range Test, $P < 0.05$). DW: dry weight.

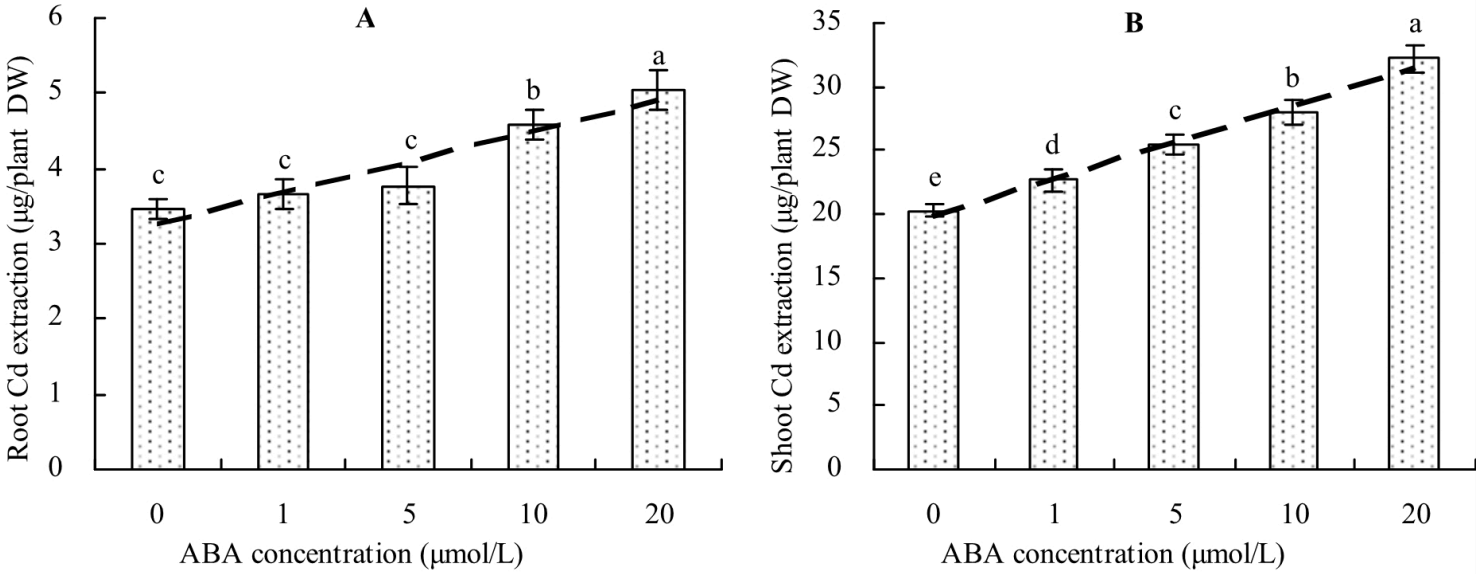


Figure 5

Cd extractionof *N. officinale*. **A**: root Cd extraction; **B**: shoot Cd extraction. Different lowercase letters indicate significant differences among the treatments (Duncan's Multiple Range Test, $P < 0.05$). Dashed line indicates the regression relationship between the ABA concentration and Cd extraction. Root Cd extraction = Cd content in roots ´ root biomass; shoot Cd extraction = Cd content in shoots ´ shoot biomass. DW: dry weight.

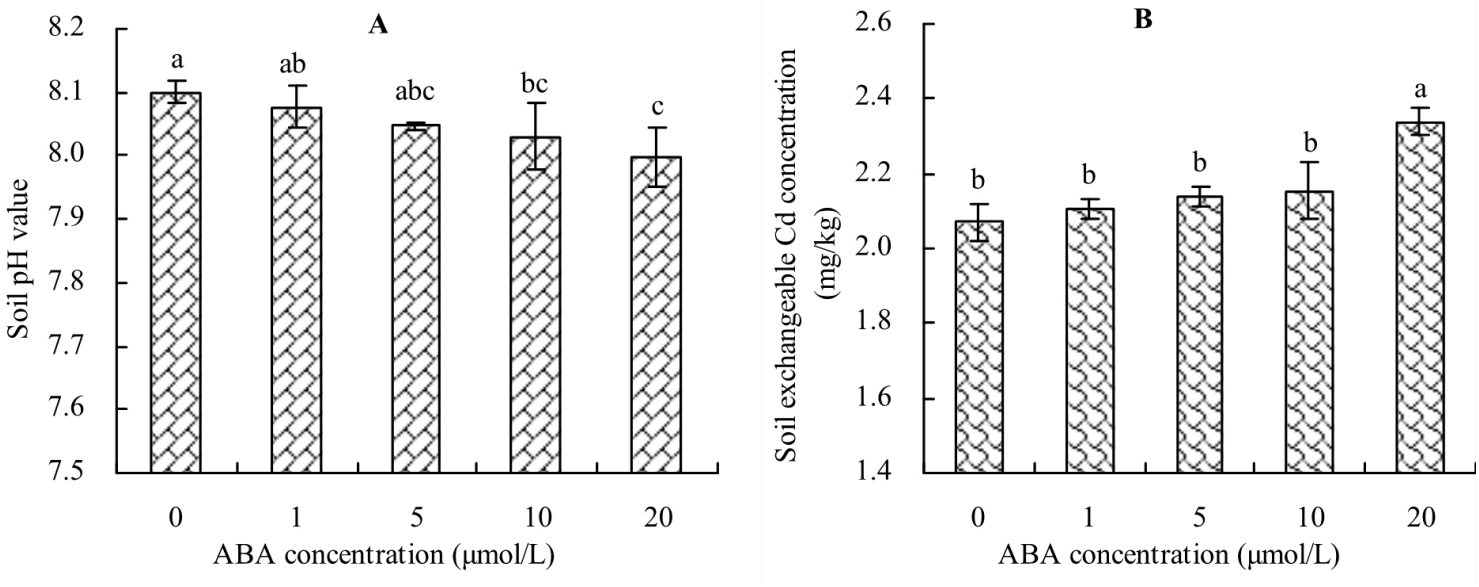


Figure 6

Soil pH value and soil exchangeable Cd concentration. **A**: soil pH value; **B**: soil exchangeable Cd concentration. Different lowercase letters indicate significant differences among the treatments (Duncan's Multiple Range Test, $P < 0.05$).

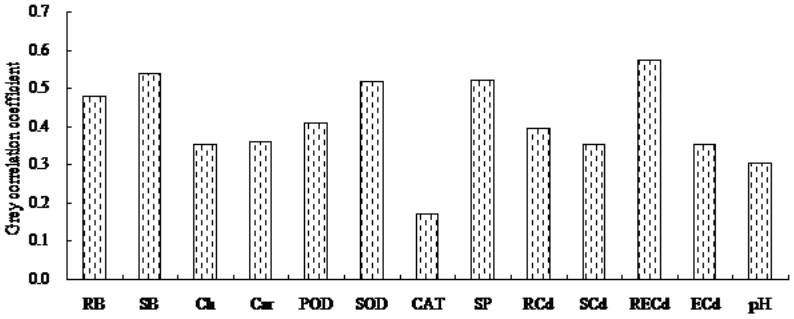


Figure 7

Grey correlation coefficients of the different indicators with the shoot Cd extraction. RB = root biomass; SB = shoot biomass; Chl = total chlorophyll content; Car = carotenoid content; POD = POD activity; SOD = SOD activity; CAT = CAT activity; SP = soluble protein content; RCd = root Cd content; SCd = shoot Cd content; RE = root Cd extraction; ECd = soil exchangeable Cd concentration; pH = soil pH value.