Chemosphere 352 (2024) 141317

Contents lists available at ScienceDirect

Chemosphere

journal homepage: www.elsevier.com/locate/chemosphere

Effect of ammonium sulfate combined with aqueous bio-chelator on Cd uptake by Cd-hyperaccumulator *Solanum nigrum* L.

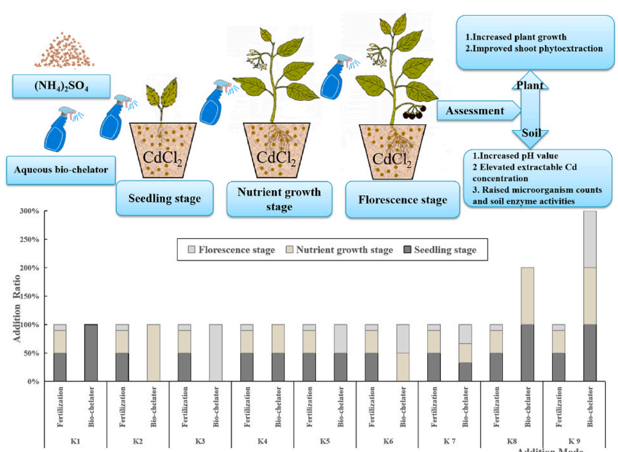
Wei Yang [a,**], Huiping Dai [b,***], Shuhe Wei [c,*], Brett H. Robinson [d], Jianming Xue [e]

[a] Academy of Environmental and Chemical Engineering, Shenyang Ligong University, Shenyang, 110159, Liaoning, China
[b] College of Biological Science & Engineering, Shaanxi Province Key Laboratory of Bio-resources, Shaanxi University of Technology, Hanzhong 723001, China
[c] Key Laboratory of Pollution Ecology and Environment Engineering, Institute of Applied Ecology, Chinese Academy of Sciences, Shenyang 110016, China
[d] School of Physical and Chemical Sciences, University of Canterbury, Christchurch 8041, New Zealand
[e] New Zealand Forest Research Institute (Scion), POB 29237, Christchurch 8440, New Zealand

HIGHLIGHTS

- ◆Co-application of $(NH_4)_2SO_4$ and bio-chelator was more effective than alone.
- ◆K8 and K9 treatments achieved the optimal Cd phytoextraction pattern.
- ◆The microorganism counts and enzyme activities advanced most for K8 and K9.
- ◆2 mg kg^{-1} Cd pollution could be removed after three rounds of phytoremediation.

GRAPHICAL ABSTRACT



ARTICLE INFO

Handling editor: Lena Q. Ma

Keywords:
Cadmium
Aqueous bio-chelator
Enhanced phytoremediation
Ammonium sulfate ($(NH_4)_2SO_4$)
Solanum nigrum L

ABSTRACT

The efficacy of using plants to phytoremediate heavy metal (HM) contaminated soils can be improved using soil amendments. These amendments may both increase plant biomasses and HMs uptake. We aimed to determine the composite effect of ammonium sulfate ($(NH_4)_2SO_4$) combined with the application of an aqueous stem-extracted bio-chelator (*Bidens tripartita* L) on the plant biomasses and cadmium (Cd) phytoextraction by *Solanum nigrum* L. The constant $(NH_4)_2SO_4$ application mode plus bio-chelator additives collectively enhanced the shoot Cd extraction ability owing to the increased plant biomass and shoot Cd concentration by *S. nigrum*. The shoot Cd extraction and the soil Cd decreased concentration confirmed the optimal Cd phytoextraction pattern in

* Corresponding author. Key Laboratory of Pollution Ecology and Environment Engineering, Institute of Applied Ecology, Chinese Academy of Sciences, Shenyang 110016, China.
** Corresponding author.
*** Corresponding author.
E-mail addresses: yangwei0709@sylu.edu.cn (W. Yang), daihp72@snut.edu.cn (H. Dai), shuhewei@iae.ac.cn (S. Wei).

https://doi.org/10.1016/j.chemosphere.2024.141317
Received 19 December 2023; Received in revised form 25 January 2024; Accepted 26 January 2024
Available online 27 January 2024

K8 and K9 treatments (co-application of $(NH_4)_2SO_4$ and twofold/threefold bio-chelators). Accordingly, Cd contamination risk in the soil (2 mg kg^{-1}) could be completely eradicated (<0.2 mg kg^{-1}) after three rounds of phytoremediation by *S.nigrum* based on K8 and K9 treatments through calculating soil Cd depletion. The microorganism counts and enzyme activities in rhizosphere soils at treatments with the combined soil additives apparently advanced. In general, co-application mode of $(NH_4)_2SO_4$ and aqueous bio-chelator was likely to be a perfect substitute for conventional scavenger agents on account of its environmental friendliness and cost saving for field Cd contamination phytoremediation by *S. nigrum*.

1. Introduction

While agricultural and industrial development has brought exceptional economic benefits in the world, it has also resulted in widespread soil contamination (Rizwan et al., 2018a). Cadmium (Cd) is a non-essential heavy metal (HM) that reduces plant growth through the inhibition ofphotosynthesis, creation ofmineral nutrient imbalances, and oxidative stress (Niu et al., 2021). Meanwhile, it is a human carcinogen, and may accumulate in the food chain (Yan et al., 2018).

Potentially, phytoextraction may be used to remove HMs, from contaminated soil (Ahemad, 2019), although there is a lacuna of examples of successful operations (Robinson et al., 2018).Hyperaccumulators, plants that accumulate inordinately-high concentrations of HMs in the aerial portions may extract HMs from contaminated soil in situ, without excessive soil disturbance (Burges et al., 2018). Hyperaccumulators can tolerate high concentrations of HMs with no obvious physiological damage (Guo et al., 2017), the HM concentrations in their aerial portions exceed the concentrations in soil (Liu et al., 2019). Depending on the HM, the threshold concentration for hyperaccumulation is a leaf concentration 10–100 times higher than the soil concentration. Typical thresholds are 100 mg kg^{-1} (for Cd, Tl), 1000 mg kg^{-1} (Ni, As, Se, B, Co) and 10,000 mg kg^{-1} (Zn, Mn) (Lu et al., 2018). Many Cd hyperaccumulators belong to the *Brassica* family or the *Brassicaceae* (*Noccaea, Arabidopsis, Rorippa*) or *Solanacea,* such as *Sedum alfredii* Hance. (Tao et al., 2020), *Sphagneticola calendulacea* (Liu et al., 2019; Lu et al., 2020). *Arabidopsis helleri* (Kushwaha et al., 2022), *Solatium nigrum* L (Dai et al., 2022), *Noccaea caerulescens* (Yan et al., 2022), *Rorippa globosa* (Dou et al., 2019), and *Lantana camara* L (Liu et al., 2019), etc.

Despite early enthusiasm, one drawback of phytoextraction is the remediation time of at least10 years or even much longer timesneeded even for moderately-contaminated soil (Zhao et al., 2015).There is an urgent needto overcome the conventional phytoremediation bottlenecksresulting from limited metal uptake by low-biomass hyperaccumulators. (Wu et al., 2020). To date, many papers and reports have demonstrated that a variety of techniques can be used to increase the efficacy of Cd-hyperaccumulator systems (Gu et al., 2022; Sahito et al., 2022), by increasing both biomass and Cd concentration (Rostami and Azhdarpoor, 2019).

This may be achieved through soil conditioners including N fertilizers (NO_3^--N, NH_4^+-N), crop straw and split N fertilization for reducing N losses could promote the aboveground biomasses and strengthen the Cd remediation efficiency by hyperaccumulators planted in the Cd-contaminated soil (Huo et al., 2018; Lin et al., 2018; Yang et al., 2020). Other studies on Cd phytoremediation research discovered that the applicationof plant growth regulators (PGRs), plant growth-promoting bacteria (PGPB) and chelating agents by leading to the exaltation of Cd bioavailability in soils elevated Cd concentration at different parts of the plants, and finally improved Cd phytoaccumulation by tested plants (Chen et al., 2020a; Pramanik et al., 2018; Chen et al., 2020b). Noteworthily, some chemical chelators, such as EDTA, EDDS and EGTA, were difficult to degrade and would cause secondary pollution for environmental security (Wang et al., 2019). Therefore, to explore a newly high-efficiency, environment-friendly and low-cost chelating agent is key to phytoremediation improver.

In their 2016 study, Wang et al. discovered that aqueous extracts from R. globosa shoots significantly increased Cd concentrations in the roots and shoots of the Cd-hyperaccumulator *Galinsoga parviflora*. This effect was partly due to an increased proportion of exchangeable Cd in soils. They concluded that natural green additives positively affect the soil rhizosphere, serving as potential alternatives to conventional chelators (Wang et al., 2016). Han et al. (2019) found that stem aqueous extracts of *Bidens tripartita* L. are cleaner chelating agents compared to EDTA for Cd extraction and accumulation in contaminated soil by *S. nigrum* (Han et al., 2019).*S. nigrum*, identified by Wei et al. (2006)as a new Cd hyperaccumulator, is highly effective in phytoremediation of slightly Cd-contaminated soil. Its advantages include large biomass, rapid growth, strong Cd accumulation and tolerance, and adaptability to various regions (Wei et al., 2006; Dou et al., 2022).

However, there has been limited detailed experimental research on the combined use of N fertilizers and bio-chelating agents to enhance phytoremediation by *S. nigrum*. The aims of our study were i) to evaluate whether the co-application of $(NH_4)_2SO_4$ and bio-chelators could enhance the shoot Cd extraction by increasing plant biomass and jointly raising shoot Cd concentration by *S. nigrum*, and ii) to explore the optimal Cd removal from contaminated soil under different co-application doses and patterns. We hypothesize that $(NH_4)_2SO_4$ combined with the double doses of aqueous bio-chelators will be considered the top co-application mode due to the highest level of shoot Cd extraction through the maximum Cd concentration in the aboveground parts of *S. nigrum*. Furthermore, this method shows great promise for practical application in the phytoremediation of soils contaminated with low to moderate levels of Cd.

2. Materials and methods

2.1. Basic physicochemical properties of soil and the pot experiment

The tested soil samples were obtained from the soil surface in a depth of 0–20 cm at the Shenyang Ecological Experimental Station of Chinese Academy of Sciences (41°31′ N and 123°41′ E), which belong to brown soils (GB/T 17296-2009). The background Cd concentration in soil was 0.16 mg kg^{-1} and the pH was 6.94. The soil contained 15.2 g kg^{-1} organic carbon, total N 0.85 g kg^{-1}, available P of 10.4 mg kg^{-1}, available K 81.0 mg $kg^{-1}$and CEC 22.9 cmol kg^{-1}, respectively.The other physicochemical properties referred to Yang et al. (2019). The pot trial was performed in the station (41°31′ N and 123°41′ E) of the semi-moist continental climates prevailing with a total annual radiation of 520–544 kJ cm^{-2} average annual precipitation of 650–700 mm, and approximately 127–164 frostless days per year (Wei et al., 2010).

In all the treatment groups, Cd was spiked in deionized water with no Cd detection and added into the prepared soil as the form of $CdCl_2$ ·2.5H_2O with 2 mg kg^{-1} concentration (exclude the CK). According to the National Soil-Environmental Quality Standard of China (NSEQSC GB 15618-2018), the soil pollution level was considered moderate (SEQ, 2018). All the soil samples of 2.5 kg DW were loaded in plastic pots (φ = 20 cm and H = 15 cm) and equilibrated for 2 months (from March 15 to May 15). Six four-leaves *S. nigrum* seedlings with uniform height were transplanted from the tray into the tested pot. Each treatment was repeated in triplicate.

2.2. Experiment design and exogenous additives preparation

The appropriate N fertilizer type and addition mode, 800 mg kg^{-1} $(NH_4)_2SO_4$ (9.4 g per pot) ((5:4:1): 50 % added at base, 40 % irrigated at nutrient growth stage and 10 % at fluorescence stage) was selected according to Yang et al. (2019, 2020)in previous studies (Yang et al., 2019, 2020). Additionally, by description of one research on enhancing Cd phytoremediation by *S. nigrum*, the optimum bio-chelator, 20 % stem extract of accumulator *Bidens tripartita* L., was designated as the target bio-chelator concentration and type in this trial (Han et al., 2019). In the present experiment, the accumulator *B. tripartita* was collected from this station, and subsequently, divided into three parts: roots, stems and leaves. The EDTA reagent and $(NH_4)_2SO_4$ were purchased from Sinopharm Chemical Reagent Co. Ltd. (China). The Cd concentrations in all the additives was <0.01 mg kg^{-1}.

The collected plant stem parts were thoroughly washed with aseptic water and dried in the oven at 105 °C for 15 min, subsequently, incubated at 70 °C overnight until reaching constant weight and completely dry condition, and then the dried plant samples were pulverized and passed through a 2 mm nylon sieve. Sieved stem samples (200g) were soaked in 1000 mL distilled water and oscillated in an oscillator at 25 °C for 24h. The resulting suspension was centrifuged at 180 r min^{-1} for 30 min (Han et al., 2019) and filtered through a 0.45 μm filter membrane (Longjin film technology Co., Ltd. (China))and the filtrate was stored in a refrigerator at 4 °C for preparation. The related organic contents of 20 % (M/V) prepared aqueous extracts, such as soluble polysaccharide, was determinated by Phenol-sulfuric acid method (Wu et al., 2016), the detection of total organic acid was described by Dobrowolskaiwanek (2015) through Scid-base neutralization titration method (Dobrowolskaiwanek, 2015), reducing sugar referred to the 3,5-Dinitrosalicylic acid method (Han et al., 2019), free amino acids were detected using Ninhydrin colorimetry method (Kong et al., 2017), and the corresponding contents was 58.1 μg g^{-1}, 12.6 %, 10.5 μg g^{-1} and 4.68 μg g^{-1}, respectively.

Twelve treatments were set up in this experiment, namely CK (without Cd and additives), CK1 (with Cd, no other additives), CK2 (with Cd and EDTA). Table 1 gives a description of the treatmentsK1 ($(NH_4)_2SO_4$ (5:4:1) + bio-chelator (100 % irrigated at base)), K2 ($(NH_4)_2SO_4$ (5:4:1) + bio-chelator (100 % irrigated at nutrient growth stage)), K3 ($(NH_4)_2SO_4$ (5:4:1) + bio-chelator (100 % irrigated at fluorescence stage)), K4 ($(NH_4)_2SO_4$ (5:4:1) + bio-chelator (50 % irrigated at base, 50 % at nutrient growth stage)), K5 ($(NH_4)_2SO_4$ (5:4:1) + bio-chelator (50 % irrigated at base, 50 % at fluorescence stage)), K6 ($(NH_4)_2SO_4$ (5:4:1) + bio-chelator (50 % irrigated at nutrient growth stage, 50 % at fluorescence stage)), K7 ($(NH_4)_2SO_4$(5:4:1) + bio-chelator (1/3 irrigated at base, 1/3 at nutrient growth stage and 1/3 at fluorescence stage)), K8 ($(NH_4)_2SO_4$ (5:4:1) + bio-chelator 2-folds content (50 % irrigated at base, 50 % at nutrient growth stage)), K9 ($(NH_4)_2SO_4$ (5:4:1) + bio-chelator 3-folds content (1/3 irrigated at base, 1/3 nutrient growth stage and 1/3 at fluorescence stage)). Among all the treatments, 9.4 g pot^{-1} $(NH_4)_2SO_4$ were supplemented with 50 % at base mixed with the tested soil in the form of particles, 40 % irrigated at nutrient growth stage and 10 % at fluorescencestage by liquid irrigation (Yang et al., 2020); The stem aqueous solutions of *B. tripartita* was added in the variable additive amounts at the different time intervals including the ratio of 5:4:1 $(NH_4)_2SO_4$ application. The applications of EDTA were irrigated at nutrient growth stage (the start after 30 day of seedling transplantation). The detailed addition modes and contents are elaborately shown in Table 1 (Table 1).

All the pot-cultured plants were randomly placed in the grid greenhouse of the station and watered with tap water twice daily in the morning and evening for keeping the soil water holding capacity at approximately 80 %. Finally, the tested plants were harvested with a 118 days of growth period, of which, the seedling stage lasts from June 1 to July 1 (30 days), the nutrient growth stage from July 2 to August 11 (40 days), the fluorescence stage from August 12 to September 29 (48 days), subsequently, were reaped thoroughly.

Table 1
Experimental treatments with exogenous additives in pot cultivation by *S. nigrum*.

Treatment	Detail information of treatment
CK	*S. nigrum* without Cd, $(NH_4)_2SO_4$ and bio-chelator
CK1	*S. nigrum* with Cd, without $(NH_4)_2SO_4$ and bio-chelator
CK2	*S. nigrum* with Cd and EDTA 2 mmol kg^{-1} (irrigated at nutrient growth stage), without $(NH_4)_2SO_4$ and bio-chelator
K1	$(NH_4)_2SO_4$ (9.4g pot^{-1})(5:4:1): 50 % added at base, 40 % irrigated at nutrient growth stage and 10 % at fluorescence stage + Mode 1 bio-chelator 125 mL pot^{-1} (100 % irrigated at base)
K2	$(NH_4)_2SO_4$ (9.4g pot^{-1})(5:4:1): 50 % added at base, 40 % irrigated at nutrient growth stage and 10 % at fluorescence stage + Mode 2 bio-chelator 125 mL pot^{-1} (100 % irrigated at nutrient growth stage)
K3	$(NH_4)_2SO_4$ (9.4g pot^{-1})(5:4:1): 50 % added at base, 40 % irrigated at nutrient growth stage and 10 % at fluorescence stage + Mode 3 bio-chelator 125 mL pot^{-1} (100 % irrigated at fluorescence stage)
K4	$(NH_4)_2SO_4$ (9.4g pot^{-1}) (5:4:1): 50 % added at base, 40 % irrigated at nutrient growth stage and 10 % at fluorescence stage + Mode 4 bio-chelator 125 mL pot^{-1} (50 % irrigated at base, 50 % at nutrient growth stage)
K5	$(NH_4)_2SO_4$ (9.4g pot^{-1})(5:4:1): 50 % added at base, 40 % irrigated at nutrient growth stage and 10 % at fluorescence stage + Mode 5 bio-chelator 125 mL pot^{-1} (50 % irrigated at base, 50 % at fluorescence stage)
K6	$(NH_4)_2SO_4$ (9.4g pot^{-1})(5:4:1): 50 % added at base, 40 % irrigated at nutrient growth stage and 10 % at fluorescence stage + Mode 6 bio-chelator 125 mL pot^{-1} (50 % irrigated at nutrient growth stage, 50 % at fluorescence stage)
K7	$(NH_4)_2SO_4$ (9.4g pot^{-1})(5:4:1): 50 % added at base, 40 % irrigated at nutrient growth stage and 10 % at fluorescence stage + Mode 7 bio-chelator 125 mL pot^{-1} (1/3 irrigated at base, 1/3 at nutrient growth stage and 1/3 at fluorescence stage)
K8	$(NH_4)_2SO_4$ (9.4g pot^{-1})(5:4:1): 50 % added at base, 40 % irrigated at nutrient growth stage and 10 % at fluorescence stage + Mode 8 bio-chelator 250 mL pot^{-1} (50 % irrigated at base, 50 % at nutrient growth stage)
K9	$(NH_4)_2SO_4$ (9.4g pot^{-1})(5:4:1): 50 % added at base, 40 % irrigated at nutrient growth stage and 10 % at fluorescence stage + Mode 9 bio-chelator 375 mL pot^{-1} (1/3 irrigated at base, 1/3 nutrient growth stage and 1/3 at fluorescence stage)

Note: Doses of $(NH_4)_2SO_4$ and EDTA are supplemented for analytically pure reagents.

2.3. Sample determination

The plant samples were divided into the root and shoot parts. Cd concentrations in plant samples after digestion with 87 %HNO_3 (analytical reagent) and 13 % $HClO_4$, (analytical reagent) purchased from Sinopharm Chemical Reagent Co. Ltd. (China), were determined by atomic absorption spectrophotometry (AAS, Hitachi 180). The available Cd concentration in the soil was extracted with 1 mol L^{-1} $MgCl_2$ for analysis by the above-mentioned instrument. The international certified standard reference materials (NISTSRM1547, GBW07405, GSS-5) were utilized as the soil and plant quality control parameters, respectively. The soil pH was determined by a pH meter (PHS–3B, purchased from Shanghai Instrument (Group) Co., Ltd. (China)) at the soil/water ratio of 1:2.5 (v/w) according to the previous measures (Yang et al., 2020). The basic properties of soil, such as the amounts of bacteria and the activities of catalase and urease were detected by the regularly used method described by the related researchers (Zhan et al., 2019).

2.4. Data processing and statistical analysis

Data processing and standard deviation (SD) calculations were performed using Microsoft Excel. Means of different treatments were evaluated using one-way ANOVA with DPS (Data Processing System) software. According to Duncan's multiple comparison, the significance level was $p < 0.05$ based on the statistical analysis principal (n = 3) (Li et al., 2018).

Shoot Cd extraction (μg plant^{-1}) = Shoot Cd concentration (mg kg^{-1}) × Shoot biomass (g plant^{-1})

Soil Cd phytoextraction efficiency (%) = [(Cd extraction contents of Root (μg plant^{-1}) + Cd extraction contents of Shoot (μg plant^{-1})] × 6 × 10^{-3}/original Cd contents of soil (mg pot^{-1})] × 100

soil Cd decreased concentration (mg kg^{-1}) = original Cd concentration of soil (2 mg kg^{-1})-Cd concentration in soil after harvested (mg kg^{-1})

Soil Cd depletion (mg pot^{-1}) = soil Cd decreased concentration (mg kg^{-1}) × soil weight (kg pot^{-1})

3. Results and discussion

3.1. Effect of $(NH_4)_2SO_4$ combined with bio-chelator on root and shoot biomasses by S. nigrum

Fig. 1A shows that the combined addition of $(NH_4)_2SO_4$ with the bio-chelator from the stem of accumulator *B. tripartita* into the Cd-contaminated soil, the Cd-hyperaccumulators *S. nigrum* root and aboveground biomasses at either treatments (K1, K2, K3, K4, K5, K6, K7, K8, K9) increased by 1.66-, 2.02 times, 1.69-, 1.99 times, 1.73-, 2.02 times, 1.69-, 2.01 times, 1.71-, 2.02 times, 1.70-, 2.03 times, 1.70-, 2.04 times, 1.69- times, 2.0 times and 1.69-, 1.99 times, respectively, compared with the control CK1 (with Cd, no other additions) ($p < 0.05$). Conversely, after applying EDTA to the Cd-contaminated soil (CK2), the biomasses at roots and aboveground parts of the Cd-hyperaccumulators *S. nigrum* significantly reduced ($p < 0.05$), compared with the control CK1 (with Cd, without other additions), the production of biomasses decreased 45.2 % and 44.3 %, respectively ($p < 0.05$).

In some previous studies, the investigators concluded that on account of the essential mineral nutrient supply, one-off or partition N fertilizer applications of different types at the appropriate plant growth period could dramatically improve the Cd phytoextraction capacity by hyperaccumulators relying on the increased biomasses (Huo et al., 2018; Yang et al., 2020). These preceding conclusions were similar with our experimental findings (Fig. 1A). Nevertheless, other researchers have confirmed that straws with maize and wheat provided some available nutrients (N, P, K and other nutrients) directly and indirectly absorbed by functional plants to satisfy the nutrient demands through short- and long-terms manners (Wu et al., 2019; Liu et al., 2019). In brief, the addition of aqueous bio-chelators alone into contaminated soil either increased the plant biomasses or elevated Cd concentration to shorten phytoremediation period by the target HMs hyperaccumulators (Rizwan et al., 2018b; Huang et al., 2019), the appearance of the dissimilarity for the above consequences was attributed to the interactions with soil-environment-rhizosphere systems (Gu et al., 2020).

In summary, our experimental results further displayed that the application of $(NH_4)_2SO_4$ supplemented with the stem-extracted aqueous bio-chelator posed a synergistic effect on strengthening Cd phytoremediation efficiency by *S. nigrum* planted in contaminated soil; Thereinto, the role of N fertilizer $(NH_4)_2SO_4$ was to increase biomasses, while the exogenous bio-chelator additives contributed to the elevated Cd concentration at roots and aboveground parts of the target plant (Table 2; Fig. 1A).

3.2. Effects of $(NH_4)_2SO_4$ combined with bio-chelator on shoot Cd extraction

Addition of $(NH_4)_2SO_4$ in combination with stem-extracted bio-chelating additives (*Bidens tripartita* L.) added to *S. nigrum* inCd-contaminated soil significantly increased both root and shoot Cd concentrations compared with the control, CK1 (Table 2).Increasing bio-chelator rates (K1–K9) resulted in increased shoot Cd concentrations up to a maximum concentration of Ca. 30 mg kg^{-1} (K8, K9), representing a bioaccumulation coefficient (BAC) of 15. Root Cd concentrations were Ca. 80 % of the shoot concentrations (Table 2).

In contrast to the control CK1, at K8 and K9 treatments the Cd concentrations in the roots and shoots, shoot Cd extraction and soil Cd phytoextraction efficiency by hyperaccumulators increased by 69.9 %, 70.6 %, 57.2 %, 58.0 %, 3.71-, 3.73 folds and 3.67-, 3.70 folds, respectively ($p < 0.05$) (Table 2). The soil Cd decreased concentration (mg kg^{-1}) and soil Cd depletion (mg pot^{-1}) were followed as K9=K8 > K7=K6=K5=K4=K3=K2=K1 > CK2 (CK1), suggesting the K8 and K9 was with the highest Cd phytoextraction efficiency due to the bio-chelator addition to the soil causing the remarkable elevation in shoot and root concentrations of *S. nigrum* (Table 2). Consequently, after mathematical calculation, Cd contamination in the soil could be completely removed and cleaned (<0.2 mg kg^{-1}) (NSEQSC GB 15618-2018) after three rounds of phytoremediation by co-application of $(NH_4)_2SO_4$ and aqueous bio-chelator by *S.nigrum*.

Pearson's correlation analysis showed that Cd concentrations in root

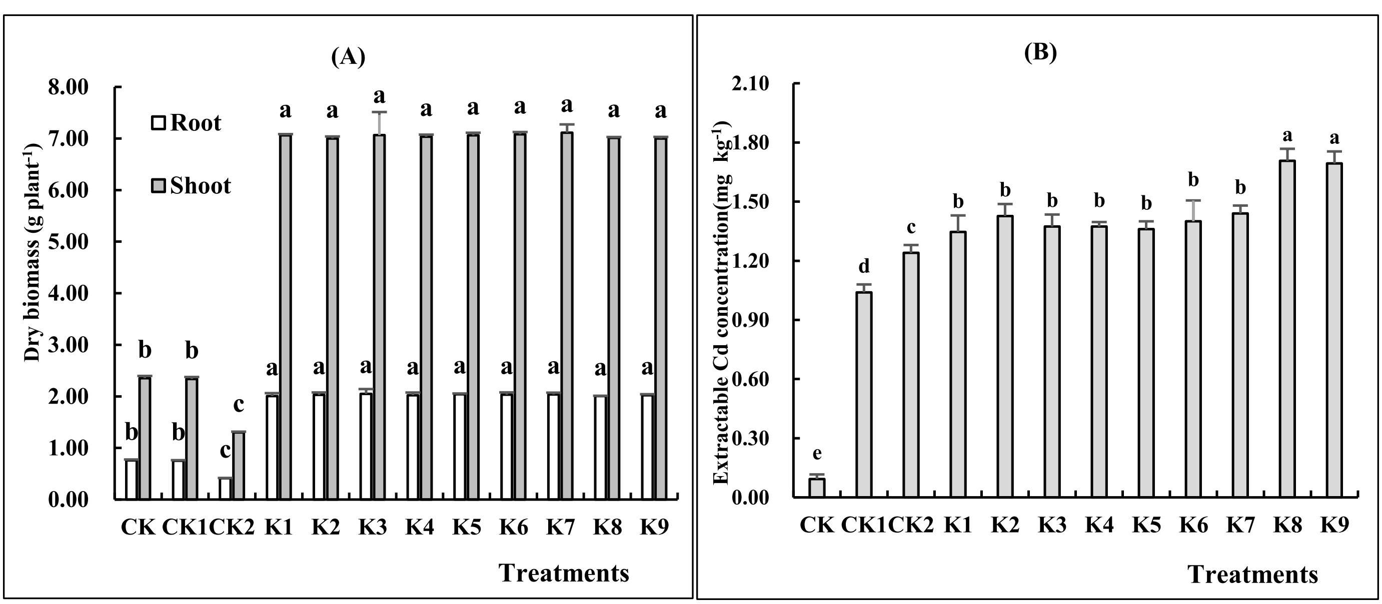


Fig. 1. Effect of $(NH_4)_2SO_4$ combined with bio-chelator on *S. nigrum* root and shoot biomass (A) and on extractable Cd concentration in soil (B) (Means with different letters among treatments were significantly different ($p < 0.05$)).

Table 2
Effect of $(NH_4)_2SO_4$ combined with bio-chelator on Cd extraction by *S. nigrum*.

Treatments	Root concentration (mg kg^{-1})	Shoot concentration (mg kg^{-1})	Shoot Cd extraction (μg $plant^{-1}$)	Soil Cd phytoextraction efficiency (%)	Soil Cd decreased concentration (mg kg^{-1})	Soil Cd depletion (mg pot^{-1})
CK	0.03 ± 0.01e	0.04 ± 0.01e	0.10 ± 0.02e	–	–	–
CK1	14.4 ± 0.71d	19.3 ± 0.51d	45.2 ± 1.82c	6.72 ± 0.17c	0.130 ± 0.004c	0.325 ± 0.011c
CK2	17.5 ± 0.88c	22.9 ± 0.65c	29.8 ± 1.17d	4.45 ± 0.18d	0.088 ± 0.008c	0.219 ± 0.020c
K1	20.9 ± 0.92b	26.4 ± 0.30b	186 ± 1.88b	27.5 ± 0.45b	0.549 ± 0.011b	1.37 ± 0.028b
K2	21.0 ± 0.71b	26.8 ± 0.89b	188 ± 7.19b	27.6 ± 0.98b	0.523 ± 0.072b	1.31 ± 0.181b
K3	20.3 ± 0.54b	27.2 ± 0.87b	192 ± 16.5b	28.1 ± 2.13b	0.560 ± 0.036b	1.40 ± 0.09b
K4	20.2 ± 0.33b	26.8 ± 0.62b	189 ± 3.38b	27.5 ± 0.26b	0.547 ± 0.006b	1.37 ± 0.014b
K5	20.5 ± 1.04b	27.1 ± 0.70b	191 ± 4.98b	28.0 ± 0.73b	0.560 ± 0.01b	1.40 ± 0.025b
K6	20.5 ± 0.65b	26.7 ± 1.42b	189 ± 11.2b	27.7 ± 1.36b	0.553 ± 0.015b	1.38 ± 0.038b
K7	21.5 ± 0.76b	27.2 ± 1.67b	194 ± 16.1b	28.5 ± 1.87b	0.570 ± 0.036b	1.43 ± 0.090b
K8	24.5 ± 1.28a	30.4 ± 0.16a	213 ± 0.70a	31.4 ± 0.32a	0.630 ± 0.01a	1.58 ± 0.025a
K9	24.6 ± 1.57a	30.5 ± 0.65a	214 ± 4.42a	31.6 ± 0.71a	0.633 ± 0.012a	1.58 ± 0.029a

Note: Means with different letters in the column are significantly different at $p < 0.05$.

and shoot portions, also shoot extraction by *S. nigrum* had significantly positive correlations ($p < 0.05$) with the bio-chelating agents addition amounts, rather than bio-chelating agents application mode (Table 4).

Our study demonstrated that combining N fertilizers with aqueous bio-chelators significantly increased the Cd uptake of *S. nigrum* (Table 2, Fig. 1A). Similar findings have been reported in other studies, where straw biochar application increased Cd accumulation in plant seedlings. This increase was attributed to increased mobility and bioavailability of Cd (Sungur et al., 2015; Gong et al., 2021). Additionally, some studies have observed that certain straws affect microbial composition (Fang et al., 2014) and produce specific chemicals that increase HM uptake by roots (Okanya et al., 2011). However, some researchers argue that straw biochar amendments reduce the bioavailable Cd concentration in contaminated soil, predominantly due to changes in soil's physico-chemical properties (Wu et al., 2019; Du et al., 2019). The lack of consensus in these findings is mainly attributed to variations in soil types, plant species, aqueous extraction methods, and plant tissues studied (Han et al., 2020).

This study revealed that in comparison to the control (CK1) with only Cd, the Cd concentrations in the roots and aerial parts of plants significantly increased in the EDTA-treated control (CK2) ($p < 0.05$). However, decreased Cd extraction was mainly attributed to a substantial reduction in plant biomass (Fig. 1A). As a prominent member of the hexahydric acid family, EDTA forms soluble metal-EDTA compounds with metals, enhancing the solubility, mobility, and bioavailability of HMs in soils. This facilitates absorption and translocation of HMs from soil to roots and shoots in plants (Gul et al., 2019; Liu et al., 2019). Numerous studies have indicated that EDTA application increases HM accumulation, thereby increasing phytoremediation efficiency (Shahid et al., 2014; Afshan et al., 2015), aligning with the findings of this study (Table 2). However, the use of chelators such as EDTA in the field has been widely discredited due to inevitable leaching losses of the chelated HM (Nowack et al., 2006).

Conversely, other research indicates that EDTA alone does not improve cadmium and other heavy metal accumulation in *S. alfredii* roots and shoots. This inefficacy is likely due to excessive EDTA adversely affecting root development and plant growth, ultimately leading to phytotoxicity (Guo et al., 2019; Li et al., 2020). Moreover, inappropriate use of EDTA in farmland soils can cause environmental issues, owing to its non-degradable nature, leading to soil physico-chemical imbalances and potential ecological risks (Guo et al., 2015).

Table 3
Effect of $(NH_4)_2SO_4$ combined with bio-chelator on microorganism counts and enzyme activities in soils.

Treatments	Bacteria number (× 10^6)	Catalase activity (mL g^{-1})	Urease activity (μg g^{-1})
CK	25.9 ± 0.4a	6.13 ± 0.12a	256 ± 11.5c
CK1	25.7 ± 0.4a	6.17 ± 0.21a	263 ± 3.3c
CK2	16.6 ± 0.9b	3.53 ± 0.25b	165 ± 1.6d
K1	25.9 ± 0.5a	6.30 ± 0.10a	331 ± 3.5b
K2	25.8 ± 0.3a	6.27 ± 0.06a	330 ± 4.6b
K3	26.4 ± 0.1a	6.13 ± 0.15a	330 ± 6.7b
K4	25.7 ± 0.6a	6.20 ± 0.17a	330.±2.6b
K5	26.1 ± 0.3a	6.13 ± 0.15a	331 ± 4.4b
K6	26.2 ± 0.5a	6.10 ± 0.10a	331 ± 5.9b
K7	26.3 ± 0.1a	6.13 ± 0.21a	334 ± 3.7b
K8	25.9 ± 0.5a	6.20 ± 0.26a	373 ± 4.6a
K9	26.0 ± 0.6a	6.13 ± 0.12a	374 ± 4.5a

Note: Means with different letters in the column are significantly different at $p < 0.05$.

3.3. Effect of $(NH_4)_2SO_4$ combined with bio-chelator on microorganism counts and enzyme activities in soil

As shown in Table 3, the addition of EDTA into the Cd-contaminated soil had a negative impact on soil environment, which caused considerable decline in bacteria amounts, catalase (CAT) activity and urease activity in the rhizosphere soil ($p < 0.05$), compared with CK1 (with Cd, no other additions) (Table 3). To the contrary, the combined application of $(NH_4)_2SO_4$ with the aqueous additives treatments did not result in the conspicuous changes in bacteria counts and catalase (CAT) activities ($p < 0.05$). Apart from this, the study demonstrated that the complex application of $(NH_4)_2SO_4$ with the natural chelating agent treatments greatly activated urease activity in the rhizosphere soil cultivating Cd-hyperaccumulators *S. nigrum* via enzyme activity assay ($p < 0.05$) (Table 3). As the bio-chelator additive contents multiplied, the soil urease activity enhanced significantly and reached to a peak, which increase by 41.9 % compared with CK1 (with Cd, no other additions) ($p < 0.05$). The above conclusions elaborated that the combined addition of $(NH_4)_2SO_4$ with the aqueous bio-chelator played a positively regulatory role on enhancing Cd phytoremediation by *S. nigrum.* Pearson's correlation analysis showed that the urease activity in soils had a significantly positive correlation ($p < 0.05$) with the bio-chelating agents addition amounts (Table 4).

Our experimental conclusions drawn in this experiment were unanimous with the results of previous study, which showed the addition of stem-extracted exogenous additives maintained and guaranteed the favorable physicochemical environment in soil-plant systems, obviously improved the living environment of micro-organisms and positively regulated microbial enzymatic activities (Han et al., 2019). Furthermore, our trial also observed that the EDTA amendment led to the physicochemical properties in soils in disorder and negatively affects microbial enzymatic activities, as another literature has reported (Vigliotta et al., 2016).

Table 4
Pearson correlation coefficients between bio-chelator with *S.nigrum* phytoremediation and soil environment.

Treatment	Root Cd concentration (mg kg^{-1})	Shoot Cd concentration (mg kg^{-1})	Shoot Cd phytoextraction (μg $plant^{-1}$)	Extractable Cd concentration (mg kg^{-1})	Bacteria number (× 10^6)	Catalase activity (mL g^{-1})	Urease activity (μg g^{-1})
Bio-chelator addition amount	0.747*	0.868*	0.863*	0.804*	0.157	0.407	0.863*
Bio-chelator application mode	0.103	0.164	0.189	0.246	0.323	0.491	0.237

Note: * is significant difference at $p < 0.05$.

3.4. Effect of $(NH_4)_2SO_4$ combined with bio-chelator on extractable Cd concentration in soil

As shown in Fig. 1B, the results explained that after the application of $(NH_4)_2SO_4$ combined with stem-extracted natural additives and the EDTA addition alone to the Cd-contaminated soil, in contrast to the CK1 (with Cd, no other additions), the extractable Cd concentration in soil significantly increased ($p < 0.05$) (Fig. 1B). As the $(NH_4)_2SO_4$ addition contents were constant and the stem-extracted exogenous additives (*B. tripartita*) contents improved to the twofold and irrigated into the soil, the extractable Cd concentrations in soil markedly elevated, which increase by 64.10 % in comparison to the CK1(with Cd and without other additions) ($p < 0.05$) (Fig. 1B). Furthermore, when the bio-chelator concentrations improved to threefold doses (375 mL pot^{-1}), the available Cd concentration did not continue to boost ($p < 0.05$) (Fig. 1B). In addition, Pearson's correlation analysis stated briefly that there was a high-positive relevance between amounts of bio-chelator additives and the extractable Cd concentration in soils (Table 4).

It is found that the mobility and availability of Cd are closely associated not only with its total concentration in Cd-contaminated soil but also with the concentration proportion of the other Cd^{2+} species including available and water soluble Cd forms in the soil (Manquián-Cerda et al., 2018). In our experiment, the circumstance of $(NH_4)_2SO_4$ supplemented with stem-extracted exogenous additives and EDTA Treatment (CK2) resulting in outstanding elevation of available Cd concentrations in contaminated soils was possibly because that the increase of active ingredients in exogenous bio-chelators immediately stimulated the dissolved organic carbon (DOC) contents in soils, which chelated with Cd, finally improved the Cd bioavailability (Wei and Twardowska, 2013). Similar research conducted on by Mousavi et al. (2021) well established that chelators like EDTA can reduce Cd toxicity by binding to toxic Cd^{2+} cations as these chelators have a high potential of binding to metals, and thereby obviously enhanced the Cd extraction by okra plants (Mousavi et al., 2021). Earlier studies pointed out that some organic acids and reducing sugars were closely involved in HMs absorption and transfer processes at aboveground parts by hyperaccumulators (Sun et al., 2006; Sheel et al., 2015), which agrees with our experimental results (Fig. 1B). Furthermore, researchers still showed that chelation was taken between amino acids extracted from xylem sap with rare metals lanthanum (La) and yttrium (Y), which improved the HMs bioavailability in soils (Wu et al., 2013).

4. Conclusions

The combined application of $(NH_4)_2SO_4$ with exogenous bio-chelator resulted in a significant increase in the plant biomasses and Cd concentrations at the root and shoot parts of *S. nigrum*, a newly Cd hyperaccumulator. Consequently, the shoot extraction capability of Cd (shoot biomass × shoot Cd concentration) by *S. nigrum* improved. As $(NH_4)_2SO_4$ content was constant and the exogenous chelating additives concentration multiplied, the Cd concentrations in the roots and shoots of *S. nigrum* obviously increased, the shoot extraction ability of Cd clearly enhanced and reached to the maximum, no longer advanced. Concurrently, the promoted circumstances appeared in extractable Cd concentration in soils and the soil microenvironment, such as the soil microbial quantity and enzyme activities in rhizosphere soils.

To sum up, Cd contamination (2 mg kg^{-1}) in the soil could be eliminated comprehensively (<0.2 mg kg^{-1}) after three rounds of phytoremediation through analysisof the shoot Cd extraction, the soil Cd decreased concentration and the soil Cd depletion. Overall, co-application mode of $(NH_4)_2SO_4$ and aqueous bio-chelator is expected to be an alternative for conventional scavenger agents in Cd contaminated soil with large scale and low level due to its advantages of environmental friendliness and high efficiency.

CRediT authorship contribution statement

Wei Yang: Writing – original draft, Data curation. **Huiping Dai:** Methodology, Formal analysis. **Shuhe Wei:** Methodology, Funding acquisition, Supervision, Project administration, Methodology. **Brett H. Robinson:** Writing – review & editing, Validation. **Jianming Xue:** Writing – review & editing, Validation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgements

This work was supported by Research Support Project for high level talent of Shenyang Ligong University, Liaoning, China (1010147001212), City-University co-construction of State Key Laboratory of Biological Resources and Ecological Environment of Qinba, China (SXZJ-2301; SXJ-2101), Innovation Capability Support Program of Shaanxi (2023WGZJ-YB-05), Scientific and Technological Innovation Team of Bioremediation and Selenium Resources Development and Utilization of Shaanxi University of Technology, and Open Fund of Cultivation State Key Laboratory of Qinba Biological Resources and Ecological Environment of Shaanxi University of Technology, China (SLGPT2019KF04-02), the project of Foreign Experts Burean of China (G2023041029L; DL2023041006L; G2022040018L).

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