Journal of Hazardous Materials 176 (2010) 269–273

Contents lists available at ScienceDirect

Journal of Hazardous Materials

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

Effect of fertilizer amendments on phytoremediation of Cd-contaminated soil by a newly discovered hyperaccumulator *Solanum nigrum* L.

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ARTICLE INFO

Article history:
Received 16 July 2009
Received in revised form
24 September 2009
Accepted 2 November 2009
Available online 6 November 2009

Keywords:
Cadmium
Fertilizer
Phytoextraction
Phytostability
Solanum nigrum

ABSTRACT

Phytoremediation is a cost-effective, simple and sustainable beneficiary technique to purify the polluted environment. *Solanum nigrum* L., a newly found cadmium (Cd) hyperaccumulator, has shown the potential to remediate Cd-contaminated soils. Present study investigated the effects of fertilizer amendments on the Cd uptake by *S. nigrum*. Chicken manure and urea are usual agricultural fertilizers and more environmental friendly. The results showed that Cd concentrations in shoots of *S. nigrum* were significantly decreased ($p < 0.05$) by 28.2–34.6%, as compared to that of without the addition of chicken manure, but not the case for urea treatment. However, Cd extraction capacities ($\mu g\,pot^{-1}$) in shoot biomass of *S. nigrum* were significantly increased ($p < 0.05$) due to increased shoot biomass. In addition, available Cd concentration in soil significantly decreased due to addition of chicken manure. Thus, urea might be a better fertilizer for strengthening phytoextraction rate of *S. nigrum* to Cd, and chicken manure may be a better fertilizer for phytostabilization.

1. Introduction

Heavy metal-contaminated soil is one of the widespread global problems. Removal of this persistent pollutant is necessary but very difficult. The remediation of large volumes of such soil by conventional physicochemical technologies previously developed for small, heavily contaminated sites would be very expensive. Phytoremediation of heavy metal-contaminated soil is an emerging technology that aims to extract or inactivate metals in soils. It has attracted attention in recent years for the low cost of implementation and environmental benefits. Moreover, the technology is likely to be more acceptable to the public than other traditional methods [1–3]. On one hand, as an important mechanism, phytostabilization can reduce ecological risk of air and water pollution of heavy metals [4]. On the other hand, phytoextraction, mainly using hyperaccumulator to remove heavy metal from polluted site, is more important approach of cleaning contaminated soil [5]. Apparently, phytoextraction is a most important way of phytoremediation to remediate polluted soil. The hyperaccumulator means a plant that can accumulate extremely high quantities of metals in its above ground biomass and its key characteristics include critical concentration property, translocation property, tolerance property and accumulation coefficient property [6].

Although there are increasing reports on discovery of hyperaccumulators, e.g. *Thlaspi caerulescens* J. et C. Presl, *Pteris vittata* L., *Sedum alfredii* H., phytoextraction technology has not widely been used in remediation practice [7–9]. The main limiting factor is the low remediation efficiency of hyperaccumulator due to limited accumulation concentration in its shoot and biomass [4]. Some researchers were dedicated to explore the mechanisms of hyperaccumulation and subsequently to improve phytoextraction efficiency by trans-gene or beneficial microorganism. Unfortunately, the progress is very low [10–12]. Thus, many studies have been focused on addition of natural and/or synthetic chelators to increase uptake and translocation of heavy metals from soil and to achieve high removal rates. Several chelating agents, such as citric acid, EDTA, CDTA, DTPA, EGTA, EDDHA, and NTA have been studied for their ability to mobilize metals and increase metal accumulation in different remediative plants [13–15]. However, the negative effects of chelators are obvious including elevated toxicity to plants

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0304-3894/$ – see front matter
doi:10.1016/j.jhazmat.2009.11.023

Table 1
Basic properties of soil and treated soil with fertilizer.

Treatment	Addition level	pH	Total Cd (mg kg^{-1})	Organic material (g kg^{-1})	Total N (g kg^{-1})	Available P (mg kg^{-1})	Available K (mg kg^{-1})
Soil	Original	6.71 ± 0.01a	0.17 ± 0.02a	17.32 ± 0.12e	0.71 ± 0.03h	10.55 ± 0.76e	88.73 ± 0.87e
Chicken manure	Original	6.95 ± 0.02a	0.21 ± 0.01a	465.27 ± 1.53a	11.79 ± 0.93a	2017.3 ± 21.75a	310.27 ± 1.25a
Soil added with chicken manure	50 g kg^{-1}	6.73 ± 0.01a	0.17 ± 0.02a	43.68 ± 0.98d	1.15 ± 0.05f	137.62 ± 1.24d	97.14 ± 0.92d
	100 g kg^{-1}	6.75 ± 0.02a	0.18 ± 0.01a	75.15 ± 1.06c	2.24 ± 0.11c	238.36 ± 2.18c	112.08 ± 1.07c
	200 g kg^{-1}	6.76 ± 0.02a	0.18 ± 0.02a	130.45 ± 3.62b	2.81 ± 0.16b	434.62 ± 3.05b	126.68 ± 1.21b
Soil added with urea	0.5 g kg^{-1}	6.71 ± 0.02a	0.17 ± 0.02a	17.78 ± 0.65e	0.96 ± 0.10g	10.29 ± 0.84e	89.11 ± 0.95e
	1 g kg^{-1}	6.72 ± 0.01a	0.18 ± 0.01a	17.72 ± 0.69e	1.23 ± 0.22e	10.33 ± 0.76e	89.09 ± 0.89e
	2 g kg^{-1}	6.71 ± 0.01a	0.17 ± 0.02a	17.75 ± 0.81e	1.35 ± 0.21d	10.31 ± 0.31e	89.01 ± 0.72e

Means in same columns followed by the same letter were not significantly different at $p < 0.05$.

and soil microorganisms and their potential risk of leaching to ground water [1,16]. Thus, the use of crop cultural technologies may be an ideal phytoextraction strengthening method through increasing plant biomass [4].

Addition of fertilizer is the most common method for increasing crop production, which is also practical for increasing plant biomass. Chicken manure and urea are two main fertilizers in agricultural production in China [17,18]. In this research, chicken manure and urea will be added to the soil to explore the effect on cadmium (Cd) accumulation by *Solanum nigrum* L. a newly found Cd-hyperaccumulator with high phytoextraction efficiency [19]. *S. nigrum* is a kind of weed species and its biomass can grow very fast after some fertilizer added, which is a better material to determine the effects of fertilizers on phytoextraction [19].

2. Materials and methods

2.1. Experimental site

All pot-culture experiments were conducted at the Shenyang Ecological Experimental Station (41°31′N and 123°41′E) which is a temperate zone with the semi-moist continental climate. The total annual radiation of the area is 520–544 kJ/cm^2. Average annual precipitation is 650–700 mm and about 127–164 days of frostless duration in a year. The background concentrations of Cd, Pb, Cu and Zn in the soil of the Experimental Station is 0.17, 15.5, 14.3 and 41.1 mg kg^{-1}, respectively, satisfying the clean soil standard for agriculture of the National Soil-Environmental Quality Standards of China (NSEQSC, GB 15618, 1995). The top 20 cm soil was used in pot study.

2.2. Cd concentration gradient experiments

To examine the effects of amendments of fertilizer on Cd phytoremediating ability of *S. nigrum* from different contaminated soils, experiments were carried out with three concentration gradients of Cd simultaneously. In the first group, no fertilizers were added. Four treatments were designed including control 1 (CK_1) without the addition of Cd, and treatments Cd_{10}, Cd_{25} and Cd_{50}, with Cd spiked at 10, 25 and 50 mg kg^{-1}, respectively. For the second group, 100 g kg^{-1} chicken manures were added into Cd treated soils, i.e. the control 2 (CK_2) added with 100 g kg^{-1} chicken manure without the addition of Cd, and treatments C_{10}, C_{25} and C_{50} with Cd spiked at 10, 25 and 50 mg kg^{-1} and added with 100 g kg^{-1} chicken manure, respectively. Similarly, in the third group, 1 g kg^{-1} of urea was added in control 3 (CK_3) as well as in treatments U_{10}, U_{25} and U_{50} spiked with Cd at 10, 25 and 50 mg kg^{-1}, respectively. Basic properties of original soil and treated soil with chicken manure and urea under 100 g kg^{-1} and 1 g kg^{-1} conditions were listed in Table 1.

Collected soil samples and fertilizers were sieved through a 4-mm sieve, then mixed with Cd added as $CdCl_2 \cdot 2.5H_2O$ according to designed concentrations and filled plastic pots (D = 20 cm, H = 15 cm), and equilibrated for 2 months.

2.3. Fertilizer concentration gradient experiments

To explore the effects of different levels of fertilizers on Cd accumulation in *S. nigrum*, experiments were conducted with different concentration gradients of two different fertilizers. The whole Cd-hyperaccumulator characteristics of *S. nigrum* were shown when treated with 25 mg kg^{-1} Cd [19]. Therefore, Cd treatment concentration under different levels of fertilizers was 25 mg kg^{-1}. Chicken manure and urea were amended at 50 g kg^{-1} (CH_{50}), 100 g kg^{-1} (CH_{100}) and 200 g kg^{-1} (CH_{200}), respectively, and 0.5 g kg^{-1} ($UR_{0.5}$), 1 g kg^{-1} (UR_1), 2 g kg^{-1} (UR_2), respectively. The control was same as the treatment in 2.2, i.e. Cd_{25}.

2.4. Plant cultivation

Seedlings of *S. nigrum* were collected from the station and transplanted into pot during spring. All pots were put in green house and the loss of water were made up using tap water (no Cd, Pb, Cu and Zn detected) to sustain 80% of soil water-holding capacity. All treatments were replicated three times. The plants were harvested after reaching their maturity.

2.5. Sample determination and data processing

Dried plant and soil samples were grinded and passed through a sieve. Samples were then digested using concentrated HNO_3 and $HClO_4$ to determine total heavy metal concentration [6]. The concentrations of extractable heavy metals in soils were extracted by using 1 mol/L NH_4NO_3 [20]. Heavy metal concentration was determined by using an atomic absorption spectrophotometer (AAS, Hitachi 180-80 with a 1.3-nm spectral band width). The measured values of heavy metals were checked by using certified standard reference material (SRM 1547, peach leaves) obtained from the National Institute of Standards and Technology (Gaithersburg, USA). The content of soil organic matter was determined by using standard methods of Lu [20]. The value of pH was determined by using a pH meter (PHS-3B), and the ratio of soil and water was 1:2.5.

The average of three replicates for each treatment and standard deviation (SD) were calculated using software of Excel and SPSS 11.5 [21]. Data were analyzed by one-way ANOVAs with the Duncan's multiple range tests to separate means. Differences were considered significant at $p < 0.05$ [21].

3. Results

3.1. Effects of same fertilizer level on the accumulation of Cd in S. nigrum

The results of three Cd concentration gradient experiments in the absence or presence of the fertilizer were showed in Table 2. Without fertilizer, the accumulation of Cd in roots and shoots of *S. nigrum* increased with the increase doses Cd. When urea was

Table 2
Effects of same fertilizer level on *S. nigrum* accumulating Cd.

Treatments	Roots (mg kg^{-1})	Shoots (mg kg^{-1})	Shoot extraction (μg pot^{-1})	Extractable Cd in soil (%)	
				Two months	Harvested time
Cd_{10}	30.5 ± 1.2a	37.8 ± 2.3a	98c	51.3a	32.5a
C_{10}	22.5 ± 1.0b	24.7 ± 1.4b	252a	32.5b	13.2b
U_{10}	31.1 ± 1.2a	38.5 ± 2.0a	242b	50.4a	33.9a
Cd_{25}	60.9 ± 2.1a	70.3 ± 3.6a	165c	52.4a	34.3a
C_{25}	45.1 ± 1.5b	47.6 ± 1.8b	480a	34.8b	15.5b
U_{25}	61.7 ± 2.7a	71.6 ± 2.9a	443b	52.1a	34.5a
Cd_{50}	98.9 ± 5.3a	106.4 ± 4.3a	177c	54.9a	35.2a
C_{50}	75.8 ± 3.6b	78.6 ± 2.6b	7514a	38.4b	17.8b
U_{50}	97.1 ± 4.3a	110.3 ± 4.5a	600b	55.3a	36.2a

Means in same Cd treatments followed by the same letter were not significantly different at $p < 0.05$.

amended at 1 g kg^{-1} to the soil, Cd concentrations in roots and shoots of *S. nigrum* was not affected (Table 2). This might be because that amendment of urea neither affect the concentrations of organic materials, phosphorous compounds or pH nor soil extractable Cd (Tables 1 and 2) [22,23]. However, shoot extractable Cd (μg pot^{-1}) was enhanced by urea amendment in most doses of Cd (Table 2).

Significant reduction of Cd concentration in roots and shoots was observed when 100 g kg^{-1} chicken manure was amended to the soil (Table 2). Soil extractable Cd was significantly lowered by the amendment of chicken manure (Table 2). The extractable Cd content was decreased 36.6%, 33.6% and 30.1% with the increase Cd doses compared to the treatments without chicken manure addition after 2 months of Cd addition. The lowest soil extractable Cd was observed in chicken manure treatments (Table 2). The differences were 59.4%, 54.8% and 49.4% as compared to the treatments without chicken manure addition in harvested time. On the contrary, significantly higher contents of the shoot extractable Cd contents (μg pot^{-1}) were obtained in this fertilizer treatment (Table 2). The decreases of extractable Cd concentrations may be caused by the chelation or consolidation of organic materials or phosphorous compounds in chicken manure [24–27]; The concentrations of organic materials and available P in the soils spiked with 100 g kg^{-1} chicken manure were 4.6 and 24.7 times higher than that of without chicken manure, respectively (Table 1).

When the plant parts were compared, regardless of the fertilizer, shoot accumulated more Cd from soil (Table 2). As a result, the aerial biomass (including shoot, leaves and inflorescence) plays a significant role in phytoextraction of Cd. To be an effective phytoremediator, the effect of Cd on growth of the aerial biomass is also critical [6]. Fig. 1 showed the combined effect of Cd contamination in the presence or absence of the two fertilizers on shoot growth of *S. nigrum*. When Cd was increased to 50 mg kg^{-1}, 37.4% reduction in shoot biomass was observed, indicating the Cd contamination was adversely affecting growth of *S. nigrum* (Fig. 1). However urea and chicken manure application significantly improved shoot growth (Fig. 1). When urea was added as the fertilizer, growth of *S. nigrum* was still sensitive to the contamination of Cd. The shoot growth was adversely retarded at 50 mg kg^{-1} Cd (Fig. 1). In contrast, when chicken manure was amended, growth retardation by Cd was not seen in all the tested doses (Fig. 1). Table 1 shows the significance increase in soil N, P and K by chicken manure. Thus this fertilizing effect may compensate or even overcome the stress by Cd in growth retardation [24–27].

3.2. Effect of different fertilizer levels on the accumulation of Cd by S. nigrum

Table 3 shows the dose effect of the fertilizer on the Cd concentration in roots and shoots of *S. nigrum*. All doses of urea in Cd-spiked soil had no effect on the Cd concentration in different organs of *S. nigrum*.

In contrast, Cd concentrations in roots and shoots of *S. nigrum* in the chicken manure treatments were significantly decreased ($p < 0.05$) (Table 3). With the increase of chicken manure content (from 50 to 200 g kg^{-1}), the further reduction in Cd concentrations was observed in roots and shoots. Shoot Cd concentration was lowest (49.8%) when 200 g kg^{-1} chicken manure was added (CH200). This reduction also correlates with the decrease in the extractable Cd in the soil presumably due to the chelation of organic materials or phosphorous (Tables 1 and 3) [24–27].

The soil fertilizing effect of urea and chicken manure was stimulating in shoot growth of *S. nigrum* (Fig. 2). The shoot growth of *S. nigrum* was doubled when treated with 1.0 g kg^{-1} urea in the presence of 25 mg kg^{-1} Cd while 2.0 g kg^{-1} urea caused a 3-fold increase (Fig. 2). Amendment of 50 g kg^{-1} or more chicken manure stimulated 3–4-fold increases in shoot growth (Fig. 2). Although urea only affects N fertility, its presence might stimulate nutrient uptake of P and K that leads general increase in growth [22–27].

The amount of phytoextracted Cd (μg pot^{-1}) was calculated by multiplying the organ Cd concentration by the organ weight and it was noted that phytoextracted Cd by *S. nigrum* in the soil amended with urea or chicken manure was increased (Figs. 1 and 2; Tables 2 and 3). The most significant effect was with 100 g kg^{-1} chicken manure.

4. Discussion

The results in this research suggest that fertilizer application can significantly increase the Cd extraction capacities of *S. nigrum*

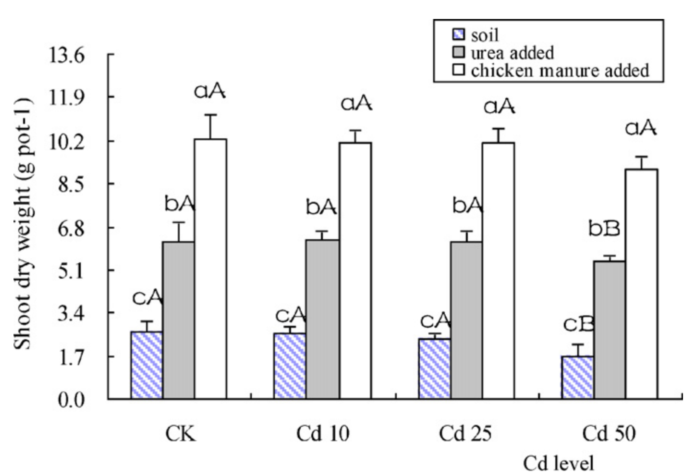

Fig. 1. Shoot biomasses of *S. nigrum* in 3 Cd concentration gradient experiments. Vertical bars represent standard deviations. The means within same Cd treatments followed by the same letter (a–c) were not significantly different at $p < 0.05$. The means among different Cd treatments followed by the same letter (A–B) were not significantly different at $p < 0.05$ either.

Table 3
Effect of different fertilizer level on *S. nigrum* accumulating Cd.

Treatments	Roots (mg kg^{-1})	Shoots (mg kg^{-1})	Shoot extraction (μg pot^{-1})	Extractable Cd in soil (%)	
				Two months	Harvested time
Cd_{25}	60.9 ± 3.0a	70.3 ± 2.8a	165g	52.4a	34.3a
CH_{50}	54.2 ± 2.3b	52.5 ± 2.0b	396d	40.2b	19.5b
CH_{100}	45.1 ± 2.6c	47.6 ± 1.7c	480b	34.8c	15.5c
CH_{200}	35.7 ± 1.2d	35.3 ± 1.8d	355e	27.5d	10.1d
$UR_{0.5}$	60.3 ± 2.9a	72.9 ± 2.9a	301f	53.1a	33.8a
UR_1	61.7 ± 3.1a	71.6 ± 2.4a	443c	52.1a	34.5a
UR_2	60.5 ± 2.6a	70.8 ± 2.8a	601a	51.8a	34.7a

Means in same columns followed by the same letter were not significantly different at $p < 0.05$.

even under the conditions of different Cd pollutant levels and fertilizer doses. The addition of chicken manure significantly increased shoot biomass of *S. nigrum* but decreased Cd concentration in its shoots due to the decrease of extractable Cd concentration in soil. The increase of Cd extraction capacity of *S. nigrum* was resulted due to increase of biomass. Although addition of urea significantly increased Cd extraction capacity of *S. nigrum*, it stimulated biomass gain instead of increasing its organ Cd concentration without affecting the extractable Cd concentration on the soil.

Usually, bioavailability of heavy metal in soil can be decreased by the organic amendment. This decreased the uptake by the transplanted plant due to changing of its available forms to some unavailable forms such as fractions associated with organic materials, carbonates or metal oxides [25]. *Chenopodium album* L. showed lower Zn concentration when some compost and manure were added [25]. Alfalfa and *Brassica juncea* L. were also reported to show similar phenomena for extracting Cd or Zn in organic material added soil [28]. Nevertheless, the organic amendment may have side effect in soil acidification which may not be desirable [26,29]. In this study, the highest tested dose of chicken manure (200 g kg^{-1}) did not further increase the shoot biomass (Table 3 and Fig. 2).

As far as our knowledge is concerned only few studies have examined the effect of using inorganic fertilizer for enhancing phytoextraction of heavy metals to clean up the soil. Our study confirm the observation made by Jalloh et al. who found that addition of urea improved the phytoextraction efficiency of Cd of rice by increasing plant biomass and maintaining a threshhold level of organ Cd content [23]. Thus, further investigation is needed for field application of the Cd hyperaccumulator in urea-amended soil.

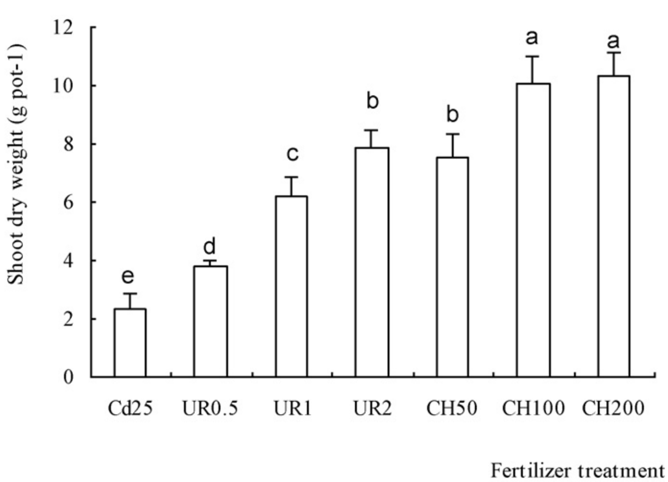

Fig. 2. Shoot biomasses of *S. nigrum* in fertilizer concentration gradient experiments. Vertical bars represent standard deviations. The means followed by the same letter (a–e) were not significantly different at $p < 0.05$.

5. Conclusion

Under pot-culture system, fertilizer addition increased the phytoextraction efficiencies of *S. nigrum* to Cd by increasing its shoot biomass. The addition of chicken manure decreased Cd concentrations of *S. nigrum*. However, urea amendment did not affect organ Cd concentration. Considering the decrease of available Cd in soil caused by chicken manure, urea might be a better fertilizer for strengthening phytoextraction rate of *S. nigrum* to Cd, and chicken manure may be a better fertilizer for phytostabilization.

Acknowledgements

This work was supported by the National Natural Science Foundation of China (Grant No. 40971184), K.C. Wong Education Foundation of Hong Kong, Natural Science Foundation of Liaoning Province of China (Grant No. 20072018), Scientific Research Foundation for the Returned Overseas Chinese Scholars of Ministry of Education of China, and Sino-Russian Science & Technology cooperation project (Grant No. 13-33).

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