1. Introduction

Heavy metal pollution of soils has dramatically increased in recent decades due to the discharge of waste and wastewater from anthropogenic sources (Ghosh and Singh, 2005). This situation has become a critical environmental issue owing to the potential adverse ecological effects of the pollutants (An, 2004). Among the heavy metals, cadmium (Cd) has been considered to be one of the most serious metal contaminants since the Itai-Itai disease reported in Japan (Yukimasa, 1975). As a non-essential element for living organisms, Cd has a very high mobility in soil-plant systems, with propensity to adversely effect both human health and the functioning of ecosystems (Perronnet et al., 2000). It is necessary, therefore, to take action towards remediation of Cd-contaminated soils.

Among the remediation techniques proposed for Cd-contaminated soils, Mulligan et al. (2001) reported success using electronic thermodynamic remediation and chemical cleanup. These techniques, however, have major drawbacks as the chemicals used result in destruction of the physicochemical properties of soils and in secondary pollution of soil and groundwater. In addition, the techniques are not suitable in situations where pollutants are present in relatively low concentrations and where the contaminated areas are large (Mulligan et al., 2001).

Compared with physical and chemical techniques of remediation, phytoremediation is a cost-effective and environmental friendly green technology that utilizes the capacity of hyperaccumulator plants to extract heavy metals from soil (Krämer, 2005; McGrath et al., 2006). Despite this, field trials or commercial operations that demonstrate successful phytoremediation of metals have been limited (Robinson et al., 2006; Maxted et al., 2007). Only Alyssum, a hyperaccumulator species for Ni phyto-remediation has been developed into a commercial technology (Chaney et al., 2007). Thus most of the hyperaccumulators tested are not commercially viable for phytoremediation (Robinson et al., 1998). Among the Cd hyperaccumulators, S. nigrum L., Populus spp, Salix/calodendron and Arabis paniculata (Wei et al., 2005; French et al., 2006; Maxted et al., 2007), may be good candidates for field conditions due to their potentially higher biomass. Furthermore, their extraction capacity may be enhanced if suitable strategies of maximizing phytoremediation are adopted, based on knowledge of good agronomic practices and management (Keller et al., 2003; McGrath et al., 2006).

To date there have been few reports on the effects of agronomic practices in field sites, for enhancing phytoremediation of heavy metal contaminated soil. Most enhanced phytoremediation studies...
have taken place on a laboratory scale with plants grown in hydroponic settings or in pot experiments with the addition of heavy metals and chelating agents, to mimic a field setting (Baker et al., 1994; Brown et al., 1995; Kayser et al., 2000; Hammer and Keller, 2003; Koopmans et al., 2008). A commonly used approach of enhancing phytoremediation has employed chelating agents such as citric acid, EDTA, DTPA and EGTA (Luo et al., 2006). However, excessive addition of chelating agents in field conditions may result in secondary pollution of soils, and the leaching of chelating agents may risk groundwater contamination as well as increase the cost of phytoremediation (Robinson et al., 2006). In studies involving Salix, Thlaspi and Arabidopsis, chemical treatments did not significantly increase the uptake of Cd and the application of EDTA decreased both biomass yield and shoot Cd concentration (McGrath et al., 2006; Maxted et al., 2007).

A comprehensive approach to phytoremediation should consider strategies in relation to the potential risk that may impact on the ecosystem (McGrath et al., 2006). In this study, a field trial was conducted using S. nigrum as a candidate hyperaccumulator of Cd. The potential of this species to extract Cd was previously demonstrated in laboratory studies and pot experiments, but had not been tested in the field. The experiments were performed in Cd-contaminated farmlands of the Shenyang Zhangshi Irrigation Area (SZIA), China, where soil had been irrigated by wastewater for more than two decades, and Cd concentrations had reached up to 10 mg kg\(^{-1}\) in surface soil. Four strategies of enhancing phytoremediation were designed to maximize the efficiency of removal of Cd from soil and based on principles of simplicity, cost effectiveness, level of efficiency and an environmentally friendly approach. The goals of this study were: (1) to investigate the phytoremediation potential of S. nigrum using strategies for enhancing phytoremediation through agronomic practice; and (2) To identify the feasibility and effectiveness of these strategies for application in Cd phytoremediation in situ on a field scale.

2. Materials and methods

2.1. Experimental site

2.1.1. Description of Shenyang Zhangshi irrigation area

Shenyang Zhangshi Irrigation Area (SZIA) is located in the western suburbs of Shenyang (122°25′–123°48′ E, 41°12′–42°17′N), Liaoning, China, where wastewater diluted by river water was used for irrigation of paddy fields. Cd contamination was first identified in 1974 when the Cd concentrations were found to be as high as 2.6 mg kg\(^{-1}\) in rice grain and up to 10 mg kg\(^{-1}\) in surface (0–20 cm) soil (Xiong et al., 2004). From 1983, the release of wastewater was gradually ceased and the more contaminated region of SZIA was rezoned from paddy field to an industrial area in 1993. However, the remaining regions are still in use as agricultural land but the farming style has been changed from paddy field rice to maize and other dry crops in order to decrease the migration of Cd in soil. SZIA is in a temperate zone with a semi-moist continental climate, with 5–9 °C as an average annual temperature, 3100–3400 °C of annual accumulative temperature above 10 °C, 520–544 kj/cm\(^2\) of total annual radiation, 650–700 mm of average annual precipitation, and between 127 and 164 frost-free days during the year.

2.1.2. Experimental site

The experimental site was a 75 m \times 20 m field located within the industrial zone close to the agricultural zone of SZIA. The soil was classified as meadow brown earth with physical and chemical properties as listed in Table 1. Individual experimental plots were distributed throughout the site, with at least 2.5 m between plots to minimise near-neighbour effects.

2.2. Seedling culture

Seedlings used in all the strategies were cultured using the following method. Seeds of S. nigrum were obtained commercially in Luoyang, Henan, China. The seedling propagation was carried out using a greenhouse-like chamber covered with polyethylene membrane and cotton quilt, which was maintained at the following conditions: natural sunlight, temperature 21/15°C (day/night); relative humidity 40–60%. Seeds were sown uniformly in the soil. Well water was added to achieve about 80% of the soil water holding capacity (WHC), which was the optimum soil moisture for seed germination as determined from preliminary tests. The seeds were incubated until six mature leaves had developed. Then, the cultivated seedlings were transplanted into the different plots to match the design planting densities of Strategies 1 to 4 as described below.

2.3. Experimental design for field trials

The field soil was ploughed and leveled to homogeneity by normal agronomic machinery prior to experimentation. Seventeen plots, each of size 6 m \times 3 m were marked out; and high ridges of 20 cm in thickness were constructed between each plot to prevent any possible interactions.

Four different strategies were employed in this field study. They were variable planting density (Strategy 1), double harvesting (Strategy 2), double cropping (Strategy 3) and the application of different fertilizers (Strategy 4). The brief parameters of the entire study are shown in Table 2 and the details of each strategy are described below.

2.3.1. Strategy 1 – planting density variation

Seedlings were transplanted into six random plots on 15th May matching the planting densities (The planting density in this work is defined as: distance between rows \times distance between plants within a row) given in Table 3. As soon as the seedlings were transplanted they were provided with irrigation using well water. During the growth stage, irrigation and hand weeding were conducted as necessary. On 7th August, plants were harvested by cutting just above the soil level. The harvested plant material was collected and taken to the laboratory for analysis.

2.3.2. Strategy 2 – double harvesting

Seedlings were transplanted into three random plots at a planting density of 50 cm \times 50 cm on 15th May and irrigated as described in 2.3.1. The plants were allowed to grow for 60 days, by which time they had reached the flowering stage, having a height of about 1 m. Two different cutting positions were selected on the basis of the foliage abundance. One group of plants was harvested by cutting at 30 cm above the soil level (named as deep cutting, DC for abbreviation) and another group was harvested by cutting at 50 cm above the soil level (mild cutting, MC), while the control plants were not cut. The plant samples from the DC and MC groups were collected and stored at 5 °C before analysis within 4 days. The remaining plants were kept growing in the field for another 60 days until they reached their mature phase. Then the 2nd harvest for the DC and MC groups was carried out by cutting at soil level as for the control plants on 14th September. Thus the DC and MC plants were harvested twice and the control plants only once.

Table 1

<table>
<thead>
<tr>
<th>Soil characteristics of the experimental site.</th>
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<td>Depth (m)</td>
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a) Hydrometer methods, CS, coarse sand (0.2–2.0 mm); FS, fine sand (0.02–0.2 mm); Silt (0.002–0.002 mm); Clay (<0.002 mm).
c) Organic carbon.
d) Method: 1 M NH\(_4\) Acetate Extractable cations with prewash.
2.3.3. Strategy 3 – double cropping

Seedling transplantation and management was as described in 2.3.2. The planting densities in the three plots were 30 × 30 cm (D1), 40 × 40 cm (D2) and 50 × 50 cm (D3), respectively. The plants were harvested (1st batch) at their mature stage. Then a second batch of seedlings which were cultured from 7th July to 7th August as illustrated in Table 2, was transplanted into the same plot at the same densities, and the harvest (2nd batch) was conducted on 15th May.

2.3.4. Strategy 4 – fertilization

Seedlings were transplanted into five plots in a planting density of 50 × 50 cm on 15th May, with the seedling transplantation and management as in Strategy 1. Two kinds of fertilizers (NH₄NO₃ and Ca(H₂PO₄)₂) were used, and 4 fertilizer treatments were designed. The type and amount of fertilizer used were as follows: Treatment 1: NH₄NO₃ only at 400 kg ha⁻¹ (F1); Treatment 2 (F2) and Treatment 3 (F3): NH₄NO₃ and Ca(H₂PO₄)₂ were mixed with ratios 2:1 (600 kg ha⁻¹; 300 kg ha⁻¹) and 1:2 (300 kg ha⁻¹; 600 kg ha⁻¹), respectively. Treatment 4 (F4) Ca(H₂PO₄)₂ only at 600 kg ha⁻¹. The final plot was a control treatment (NF) with no fertilizer. Fertilizer was applied in two stages; 40% of the total amount was applied when the seedlings were transplanted into the field; the other 60% was applied at the inflorescence phase. The cadmium content of the fertilizers used was found to be insignificant. The plants were harvested on 7th August by cutting just above the soil level.

2.4. Sampling pretreatment and Cd analysis

2.4.1. Sampling pretreatment

Plant samples taken from the plots in the field were rinsed with tap water to remove the surface dirt, carefully washed with deionized water, and separated into roots, stems and leaves. The fresh plants were weighed, and then oven-dried at 105 °C for 30 min, then at 70 °C until constant weight was obtained. For each plot, the ratio of fresh weight to dry weight of samples was determined. The dried samples were homogenised in preparation for Cd analysis.

2.4.2. Cd analysis

An accurately weighed 0.5 g sample of dried plant material was put into a 100 ml beaker covered with a funnel and 15 ml of solution containing 87% of concentrated HNO₃ and 13% concentrated HClO₄(v/v) was added. The sample was digested at 50–80 °C overnight, and then at 180 °C for 8 h to near dryness and allowed to cool. The digested solution was diluted with 2% (v/v) HNO₃ to a volume of 25.0 ml before filtration. The concentration of Cd in the digested plant sample was determined using a flame Atomic Absorption Spectrometer (AA-400, PerkinElmer, USA) at wavelength of 228.8 nm.

2.5. Statistical analysis

All experimental results were statistically analyzed using the SPSS 16.0 package. Data in the text and tables were expressed as means ± standard deviation, and error bars in the figures indicate standard deviations. The statistical significance of the differences between groups was evaluated by analysis of variance (ANOVA), and compared using least significant differences (LSD) at p < 0.05, n = 6. The Pearson correlation was calculated to examine the relationships with 95% confidence intervals.

3. Results and discussion

Table 3 lists the results obtained for plant biomass and Cd concentrations for S. nigrum at the 6 different planting densities used in plots D1 – D6. The planting densities correspond to a range of approximately 3 plants per m² to 11 plants per m², with variable plant and row spacings to enable examination the effects of these factors.

3.1. Effects of planting density on plant biomass and Cd uptake

The average root length ranged from 21.7 cm to 25.4 cm, and the plant height was between 179 cm and 217 cm. Significantly, a positive correlation was observed between root extent and biomass per single plant (r = 0.551). As roots transport nutrition and water to aboveground plant tissues (Hodge, 2004) it would be expected that the larger roots were present in plants of high biomass. Table 3 also indicates that planting density had a significant influence on biomass; for example, the dry biomass per single plant at the lowest planting density (D6) was 410 ± 10 g but was only 197 ± 22 g at the highest density (D1). Statistical analysis shows a significant negative correlation between biomass per single plant and planting density (r = −0.884). This may be caused by the competition of plants for nutrition and water.

However, there was a positive correlation between the total biomass per plot and planting densities (r = 0.991). For example in the highest density plots (D1) although dry biomass per single plant was only 197 g, the total biomass of the D1 plot was up to 43.3 kg, equivalent to 241 t ha⁻¹. In contrast, in the lowest density plots (D6), the biomass per single plant was up to 411 g, but total biomass per plot was only 24.7 kg, or 13.7 t ha⁻¹; which was only 57% of the D1 plot. This indicates that both the planting density and...
the total biomass per unit area should be considered to achieve consistently high remediation efficiency.

Cd concentrations in the aboveground plant tissue of all samples from D1 to D6 were between 9.92 mg kg\(^{-1}\) and 9.66 mg kg\(^{-1}\) (Table 3) and no significant difference was detected, indicating that the planting density had no significant effect on the uptake and accumulation of Cd by \(S. \text{nigrum}\). This is to be expected, as Cd concentrations in plant tissue depend on both the genetic predisposition of the individual plant and the contamination levels of soil (Ma et al., 2001; Shah and Nongkynrih, 2007). This situation is in contrast to that seen in laboratory experiments investigating the mechanisms of heavy metal accumulation where there is a gradient in metal concentration between the soil or growth matrix and the plant (Wójcik et al., 2005; Fayiga and Ma, 2005; Koopmans et al., 2008). Thus it is not unexpected that our results showed no Cd concentration differences in relation to planting density.

As there was no significant difference between Cd concentrations in plant tissue, the total amount of extracted Cd by the plant should be mainly determined by plant biomass. The results showed a significant correlation \((r = 0.994)\) between the total amount of extracted Cd and plant biomass, and also between the total amount of extracted Cd and planting density \((r = 0.974)\). The amount of extracted Cd per plot in the highest density plot D1 was 420 mg, equal to 0.23 kg ha\(^{-1}\), 1.62 times higher than in the lowest density plot D6, which was only 260 mg, or 0.14 kg ha\(^{-1}\) (Table 3).

Additionally, regardless of the density, the amount of Cd extracted by \(S. \text{nigrum}\) is noteworthy in comparison to other species. The non-hyperaccumulator maize grown in a polluted soil extract Cd with a pollution level of 5.0 and 0.059 kg ha\(^{-1}\) (Wójcik et al., 2005; Fayiga and Ma, 2005; Koopmans et al., 2008). This is thus not unexpected that our results showed no Cd concentration differences in relation to planting density.

Fig. 1. Dry biomass of harvested Solanum nigrum L. at different cutting positions (DC: deep cut at 30 cm above the soil level, MC: mild cut at 50 cm above the soil level and NC: no cutting). Different letters above the error bars indicate significant differences of total harvested biomass at \(p < 0.05\), \(n = 6\) for both DC and MC compared to NC. Error bars represent the standard deviation.

![Fig. 1](image1)

![Fig. 2](image2)

Fig. 2. Cd concentrations in harvested aboveground part of Solanum nigrum L. at different cutting positions. (DC: deep cut at 30 cm above the soil level, MC: mild cut at 50 cm above the soil level and NC: no cutting). DC, MC and NC show no significant differences \((p < 0.05)\), \(n = 6\). Error bars represent the standard deviation.

As illustrated in Fig. 1, the dry biomass per single plant obtained at the 1st harvest was 170 g for DC (above 30 cm), and 153 g (above 50 cm) for MC. At the final harvest, an additional 185 g for DC and 211 g for MC were obtained, so the total biomass obtained from the two harvests was 355 g in the DC group and 364 g in the MC group.

The biomass of NC was 320 g. The total biomasses obtained from MC and DC groups were 11.9% and 9.9% respectively higher than that of NC group, with mild cutting achieving the highest total biomass, similar to previous work on \(S. \text{nigrum}\) by Pei et al. (2007). The Cd concentrations in the aboveground plant tissue in the 1st harvest were 8.25 mg kg\(^{-1}\) for the DC group and 8.04 mg kg\(^{-1}\) for the MC group (Fig. 2), which were 87% and 85% respectively of the NC group concentration (9.47 mg kg\(^{-1}\)). This result was similar to previous work, in which the concentrations of Cd in the stems and leaves of \(S. \text{nigrum}\) harvested at the flowering stage were 83.1% and 85.5% of those at the mature stage (Wei et al., 2006). The Cd concentrations at the 2nd harvest were 9.63 mg kg\(^{-1}\) and 9.92 mg kg\(^{-1}\) in the DC and MC groups respectively (Fig. 2), indicating that cutting may enhance the uptake of Cd by \(S. \text{nigrum}\), even though the difference is not statistically significant in our experiment. But, in a previous study conducted in a pot experiment, the enhancement was significant (Pei et al., 2007), illustrating the point that laboratory experiments may not be representative of field trials.

The total biomass of each plot collected from the DC group was 28.6 kg (15.9 t ha\(^{-1}\)); 13.4 kg from the 1st harvest and 15.2 kg from the 2nd harvest. From MC the total biomass collected was 30.0 kg (16.7 t ha\(^{-1}\)), comprising 11.9 kg from the 1st harvest and 18.1 kg from the 2nd harvest. The total biomass from NC (control) was 25.8 kg (14.3 t ha\(^{-1}\)). The increases in sum of the biomasses from DC and MC groups were 11.0% and 16.5% respectively higher than NC (control) Group. Therefore we can conclude that double harvesting...
is an effective way of improving the biomass of \textit{S. nigrum} in field conditions.

Based on the total biomass obtained and Cd concentrations in the aboveground part of the plant, the total Cd extracted from a plot by the DC group was 257 mg (equivalent to 0.143 kg ha$^{-1}$), as 111 mg from the first harvest and 146 mg from the second harvest; similarly, the total Cd extracted by the MC strategy was 276 mg (0.153 kg ha$^{-1}$), as 96 mg from the first 180 mg and second harvests respectively. Therefore the total Cd extracted from harvested plants of each plot in the two harvests combined was 1.04 and 1.13 times that of the control, thus was slightly, but not significantly greater than the control. Therefore, it seems that harvesting twice a year was an effective way of improving the phytoremediation efficiency of \textit{S. nigrum}, but cutting position is also important and appropriate studies would be required to determine the optimum.

### 3.3. Effect of double cropping on biomass and Cd extraction

The biomasses of a single plant of the 1st batch were 242.23 g at D1, 399.41 g at D2 and 416.11 g at D3; and for the 2nd batch were 105.05 g, 138.60 g and 198 g in D1, D2 and D3, respectively (Fig. 3). The biomasses from the 1st batch at all 3 densities were significantly higher than those from the 2nd batch. This result can be explained in relation to climate characteristics in northeast China. In the autumn of the season concerned, the weather turned cold quickly, and only one month remained for the growth of plant after the 2nd batch of seedlings was transplanted. Based on this observation and data from another study (Wei and Zhou, 2006), the flowering stage was around 80% of the mature stage biomass. The biomass of the 2nd batch would have been improved if the harvesting of the 1st batch had been advanced to the flowering stage, and this will be tested in a future study.

Although \textit{S. nigrum} is an annual plant, its growth duration from seedling-transplanted to mature phase is just 2 months. Since the frostless period at the experimental site was longer than 5 months, double cropping is therefore feasible and would be an effective way to increase the efficiency of Cd-removal by \textit{S. nigrum}.

The total biomass in the D1 plots was 75.9 kg (equal to 42.1 t ha$^{-1}$), in which 53.4 kg came from the 1st batch and 23.5 kg from the 2nd batch. Similarly the total biomass of D2 plot was 69.4 kg (35.6 t ha$^{-1}$), (51.4 kg + 18.0 kg) and for D3, 51.9 kg (28.9 t ha$^{-1}$), (35.0 kg + 17.0 kg).

The Cd concentrations in the 2nd batch were higher than those in the 1st batch at all 3 densities. As is shown in Fig. 4, Cd concentrations in the aboveground part of plants in the 1st batch were 9.30 mg kg$^{-1}$, 9.16 mg kg$^{-1}$ and 9.27 mg kg$^{-1}$ in D1, D2 and D3 respectively, and were 9.69 mg kg$^{-1}$ (D1), 9.26 mg kg$^{-1}$ (D2) and 9.73 mg kg$^{-1}$ (D3) in the 2nd batch.

Based on these biomass and Cd concentrations, the amount of Cd extracted from each plot in the 1st batch was 497 mg in D1, 471 mg in D2 and 324 mg in D3; and in the second batch (228 mg, 167 mg and 165 mg in D1, D2 and D3). The sum of the extracted Cd from the 3 plots was 724 mg (0.402 kg ha$^{-1}$ D1), 638 mg (0.354 kg ha$^{-1}$ D2) and 489 mg (0.272 kg ha$^{-1}$ D3), which were 1.72, 1.83 and 1.63 times respectively higher than those with same density design but only one harvest (Table 3). So, we can conclude the phytoremediation efficiency can be significantly enhanced by twice yearly harvesting.

### 3.4. Effect of fertilization on biomass and Cd extraction

The biomass and Cd concentrations in \textit{S. nigrum} harvested following fertilizer treatments were measured. Fertilization had no effect on the biomass recovered (Fig. 5). The Cd concentrations in 2 treatments (F3 and F4) were significantly lower than that of NF (unfertilized) plants, while the F1 and F2 Cd concentrations were not significantly different to NF (Fig. 6). This indicated fertilization has played no role in increasing the biomass of \textit{S. nigrum}, and did not enhance Cd accumulation in plants.
In this study, Ca(H₂PO₄)₂ had a negative effect on Cd uptake in S. nigrum both by itself and combined with nitrate fertilizer. A greater reduction was found in the single application than in the combination. This is consistent with previous reports, where phosphate decreased the Cd uptake in plants because of the formation of metal-phosphate mineral (Longanathan and Hedley, 1997; Kirkham, 2006), reducing the solubility and mobility of the heavy metal in soil and thus inhibiting the accumulation (Berti and Cunningham, 1997). Therefore, fertilizing may not be an appropriate way of enhancing remediation of Cd in agricultural soil, and phosphate fertilization is likely to be detrimental to the remediation.

4. Conclusions

Field trials contribute practical information towards the development of phytoremediation strategies that cannot be provided by laboratory tests. The results from our field study have shown that S. nigrum has a potential application for phytoextraction of Cd from contaminated soils. Significantly, S. nigrum can accumulate Cd from soils where the concentrations are relatively low and thus it has application for use for decontamination of slightly to moderately Cd-contaminated soil. Most importantly, S. nigrum has a relatively high biomass yield compared with other known hyperaccumulating plants such as T. caerulescens and A. halleri and our study showed that biomass was the most critical determinant of Cd accumulation. We employed several agronomic practices and study showed that biomass was the most critical determinant of Cd accumulation. Significantly, Cd uptake in plants because of the formation of metal-phosphate mineral…

Acknowledgements

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