Phytoremediation and Accumulation of Cadmium from Contaminated Saline Soils by Vetiver Grass

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A soil-culture study was conducted to investigate the phytoextraction of cadmium (Cd) (20, 60, and 100 mg/kg) in two species of upland and lowland vetiver grass (Vetiveria zizanioides and V. nemoralis) with salinity levels of 1,000 mg/kg NaCl salt for 2 months. The two species of grass were highly tolerant to Cd and salt with little adverse effect on growth. Cd and salt treatments imposed significant negative effects on root length, shoot height and total dry biomass. Cd accumulation in the roots and shoots all increased significantly with increasing Cd concentration. The combined treatments of Cd and salt showed the highest root Cd accumulation in V. nemoralis (226–862 mg/kg) at Cd concentrations ranging from 20 to 100 mg/kg. Salt did not affect the accumulation of Cd but decreased the root-to-shoot Cd translocation. This was confirmed by the bioconcentration factor in root > 1 and the translocation factor < 1, which indicated the plant’s suitability for phytostabilization of Cd under saline conditions. The experiment pointed out that V. nemoralis was a better accumulator of Cd than V. zizanioides.

Keywords: cadmium, phytoextraction, phytoremediation, soil salinity, vetiver grass

INTRODUCTION

Salinity is one of the major causes of environmental stress with over 800 million hectares of land globally are salt-affected, causing great losses in agricultural productivity and the affected area is increasing day by day (Ledesma et al., 2016). Soil contamination with heavy metals is becoming increasingly serious in the world, including saline soils. Many salt marshes and farmland in arid and semiarid regions, including irrigation water in arid regions around mining areas are affected by salinity and polluted by heavy metals. Cadmium (Cd) is one of the most toxic heavy metals. It has been widely dispersed into the environment through the air by mining and smelting as well as by other man-made routes. Cd contamination of soil is a widespread problem in some parts of northern Thailand. The largest reported Cd deposits are situated in Mae Sot district, Tak province. The results of the International Water Management Institute (IWMI) survey of Cd levels in agricultural soils were presented in 2000, revealing that the soil Cd concentration ranged from 3.4 to 284 mg/kg (Unhalekhaka and Kositanont, 2008), whilst the regulatory limit for Cd and compounds in agricultural soil in Thailand did not exceed 37 mg/kg (Pollution Control Department, 2004)

Recently, Cd contamination of soil with high salinity levels was a serious problem for the remediation of many mined areas in arid regions. Phytoremediation is the use of plants and root-associated bacteria to remove, transform, or assimilate toxic chemicals located in the soils, sediments, ground and surface water, and even the atmosphere (Susarla et al., 2002). Phytoremediation has generated a great deal of interest as a cost-effective plant-based technology for the removal of toxic heavy metals from contaminated soil under natural field and greenhouse conditions (Jabeen et al., 2009). However, salinity is one of the most important environmental factors limiting phytoremediation because most of the crop plants were sensitive to salinity in soil. Plant properties important for phytoremediation under soil salinity include salt tolerance and contaminants removal. In addition, the use of plants with high tolerance to pollutants and large biomass was found to be advantageous. Contaminated saline soil limits the efficiency of phytoremediation both from heavy metal toxicity and potential effects of salt stress, and salt toxicity to heavy metal accumulation in plants. Moreover, the presence of salt caused a marked reduction in plant production, total chlorophyll content in plant tissues, and a high accumulation of proline.

There are few studies, however, of the phytoremediation of saline soil contaminated with heavy metals. Halophytes are expected to have a better tolerance against environmental stresses, such as heavy metals in comparison to salt-sensitive crop plants. Likewise, halophytes can be exploited as a significant and major plant species bearing the potential capability for desalination and the restoration of saline soils and phytoremediation alike. However, several halophyte species had rather limited uses. This is because most halophytes showed low biomass production, a shallow root system and the technology for large-scale cultivation has not been fully developed (Saifullah et al.,

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Many species of vetiver grass are potential plants for phytoremediation. They are distinguished by a fast growth rate, a well-developed root system, a large biomass and a long-term growth cycle. In addition, they showed a capability to tolerate and accumulate Cd at high concentrations (Roongtanakiat and Chairoj, 2001; Atagana, 2011; Jam- pasri et al., 2017). In the present study, two vetiver grass species, Vetiveria zizanioides and V. nemoralis were tested for Cd phytoextraction potential in saline soil. The aim of this study was to compare the tolerance of V. zizanioides and V. nemoralis to spiked contaminated soils with Cd and NaCl, and investigate the suitability of the two plant species for phytoremediation.

MATERIALS AND METHODS

Plants materials and soil preparation

Two upland and lowland vetiver grass species, V. nemoralis A. Camus (Ratchaburi ecotypes) and V. zizanioides Nash (Songkhla 3 ecotypes) were used in the study. The seedlings were obtained from the Khao Changnoom Royal Soil Remediation Study Center, Photharam District, Ratchaburi Province, Thailand, and grown in the greenhouse until they were one year old. They were acclimatized to the natural sunlight condition for at least four weeks before the experiment (Jampasri et al., 2010).

Uncontaminated soil was obtained from the Chatukak Market, Bangkok, Thailand and ground into fine powder. It was analyzed for pH, CEC, texture, organic matter, electrical conductivity and available Ca, P, K, and Mg. The experimental soils (saline and non-saline) had a natural supply of basal nutrients without adding mineral fertilizer, and the soil characteristics are given in Table 1. Both soils had a neutral pH (6.9–7.1). The soil texture was sandy clay loam with high levels of organic matter (7.02–8.19 %) and the CEC values were 27.20 cmol/kg. In the soil background, the amount of essential nutrients was relatively high, especially the amount of available phosphorus (2,609–2,760 mg/kg) and potassium (2,351–2,411 mg/kg). Saline soil had an EC value of 4.87 dS/m while the EC value of non-saline was 1.35 dS/m. Cd was applied to soil background, the amount of essential nutrients was relatively high, especially the amount of available phosphorus (2,609–2,760 mg/kg) and potassium (2,351–2,411 mg/kg). Saline soil had an EC value of 4.87 dS/m while the EC value of non-saline was 1.35 dS/m. Cd was applied to soil.

Experimental design

There were 7 different treatments: control with uncontaminated, non-saline soil (C), Cd non-saline soils at 20, 60, 100 mg/kg (C1, C2, C3), Cd saline soils at 20, 60, 100 mg/kg (T1, T2, T3) (Table 2). Four kg of air-dried soil for each treatment was thoroughly mixed in a plastic pot. For each soil type, separate trials were conducted in a completely randomized design. A healthy uniform vetiver grass was planted in a plastic pot (26 cm in height and 19 cm in diameter) and each replication consisted of one pot containing one plant.

All plant treatments were conducted in triplicate and the experimentation was performed in a greenhouse (28–32 °C, natural sunlight, 12/12 hours photoperiod, 60% relative humidity). Plants were watered every alternate day (Basumtary et al., 2012). They were harvested after 60 days of treatment and analyzed for growth and Cd accumulation. Plant samples were thoroughly washed with tap water and deionized water, separated into shoots and roots, and oven-dried (65°C for 72 hours) to determine the biomass. The stem height and root length were also measured.

Cd analysis

Plants were ground into powder and put through a 2-mm mesh sieve. 0.5 g of plant samples was acid digested in a microwave with nitric acid (HNO₃) (US EPA, 1998). The total concentrations of Cd in plants were determined by a flame atomic absorption spectrophotometer (FAAS, Varian AA-220FS). Standard solutions of metals (Merck) were used as reference. All chemicals were of an analytical grade.

Phytoremediation potential

The phytoremediation potential of grasses was evaluated using dry biomass, bioconcentration factor (BCF) and 7. Available potassium (mg/kg)
translocation factor (TF).

$BCF = \frac{\text{Cshoot or Croot}}{\text{Csoil}}$

Cd translocation from roots to shoots was measured using the TF, which was calculated as follows (Cui et al., 2007).

$TF = \frac{\text{Cshoot}}{\text{Croot}}$

Where Cshoot (mg/kg), Croot (mg/kg) and Csoil (mg/kg) represent the Cd concentrations in the shoot (stem and leaf tissues), in the root and in soil, respectively.

Statistical analysis

All experimental data were analyzed statistically using an analysis of variance (ANOVA) in the SPSS-17.0 statistical software package (SPSS, Inc.) for Windows. The difference in means was made using the least significant difference (LSD) at $P < 0.05$.

RESULTS AND DISCUSSION

Effect of Cd on plant growth

After being grown for two months in contaminated saline soil, V. zizanioides and V. nemoralis did not show any phytotoxic symptoms in all treatments. They maintained 100% survival for the whole study period. The root length and shoot height were more sensitive to Cd than the total dry biomass, and they were significantly inhibited ($P < 0.05$) by all Cd concentrations without the addition of salt (C1-C3 only).

For V. nemoralis, there was a significant difference ($P < 0.05$) in terms of root length and shoot height among various Cd treatments and the control. However, the total dry biomass of V. nemoralis showed no significant difference ($P > 0.05$) from that of control. Similar trend was observed in V. zizanioides. The negative effect of Cd contamination on the growth of V. zizanioides was obviously significant ($P < 0.05$) and the reduction of root length and shoot height was observed in Cd treated soil than the control. While Cd did not have any significant effect on the total dry biomass of V. zizanioides when compared with the control. According to all growth indices, they indicated that Cd stress affected the root length and shoot height of both species of plants.

All Cd levels negatively influenced growth of Vetiveria spp. Both ecotypes of vetiver grass were highly tolerant to Cd with little adverse effects on root length and shoot height at 20, 60 and 100 mg/kg Cd. These results are similar to the findings of Chen (2000), who found that the high metals contents limited the growth of vetiver grass during the first year, but the effect was reduced in the second year. Cull et al. (2000) conducted a similar experiment on the effect of heavy metals on vetiver growth. They also found that vetiver was highly tolerant to heavy metals and the toxicity level of Cd for vetiver grass was 45 mg/kg. Tolerance to high Cd concentrations in vetiver grass may be defined as the ability to avoid or limit the harmful effects of Cd. To overcome the effect of heavy metal, many major factors can involve in mitigating the toxic effects i.e. an ‘avoidance’ or limited translocation to the shoots, a protection of sensitive structures in the cytoplasm either by immobilization of metals in the cell wall (Rout and Das, 2003).

Combined effect of Cd and salt on plant growth

The results showed that vetiver grass can grow in soil with extremely high Cd concentration and moderate salinity. There was a significant ($P < 0.05$) decrease in all growth parameters when compared to control (Table 3). Root length, shoot height and total dry biomass of Vetiveria spp. gradually decreased with the increase in Cd concentration under salinity, and there was an inhibitory effect on growth caused by Cd and salt ($P < 0.05$). Under Cd and salt stress, the lowest decrement of dry weight (11.61 g) and shoot height (28.87 cm) of V. zizanioides occurred in T3 treatment (100 mg/kg Cd and 1,000 mg/kg NaCl) after 60 days of exposure.

Table 3  Dry biomass production (DW, g/plant), the root length (cm) and the shoot height (cm) of plants grown in various soil treatments for sixty days (mean± SE, n = 3). The different letters in each row represented significant differences ($P < 0.05$). The percentage (%), % symbol indicated the ratio of root or shoot biomass to the total biomass of the plants.

| Treatment        | Root biomass | Shoot biomass | Total biomass | Root length | Shoot height |
|------------------|--------------|---------------|---------------|-------------|--------------|
| V. nemoralis     | g            | %             | g             | %           | g            | cm          |
| (Ratchaburi ecotypes) | C 0.05±0.40  | 30.14         | 11.79±0.23    | 69.80       | 16.89±0.64a | 19.36±2.53a | 78.32±1.52a |
| C1               | 4.82±0.40    | 30.86         | 10.80±0.21    | 69.14       | 15.62±0.31  | 8.28±1.17   | 69.49±4.65a |
| C2               | 4.81±0.22    | 34.68         | 9.06±0.10     | 65.32       | 13.87±0.12  | 7.67±0.39   | 36.00±0.19  |
| C3               | 5.02±0.01    | 34.31         | 9.61±0.33     | 65.29       | 14.63±0.35  | 7.89±0.82   | 39.14±1.22  |
| T1               | 4.13±0.13    | 32.49         | 8.59±0.34     | 67.58       | 12.71±0.32  | 8.83±1.07   | 35.15±2.05  |
| T2               | 4.20±0.11    | 35.26         | 7.71±0.26     | 64.74       | 11.91±0.37  | 10.83±0.32  | 29.95±0.31  |
| T3               | 4.22±0.10    | 35.17         | 7.78±0.32     | 64.83       | 12.00±0.29a | 10.47±0.75a | 29.83±1.17  |
| V. zizanioides   | g             | %             | g             | %           | g            | cm          |
| (Songkla 3 ecotypes) | C 4.97±0.03  | 31.58         | 10.76±0.25    | 68.36       | 15.74±0.24a | 14.27±0.59   | 68.06±3.89  |
| C1               | 4.72±0.17    | 33.00         | 9.58±0.34     | 66.99       | 14.30±0.45  | 8.37±1.35   | 56.04±7.71  |
| C2               | 4.69±0.19    | 34.43         | 8.93±0.87     | 65.57       | 13.62±0.75  | 7.81±0.88   | 42.27±0.83  |
| C3               | 4.86±0.06    | 33.66         | 9.58±0.37     | 66.34       | 14.44±0.34  | 8.25±1.20   | 39.95±0.92  |
| T1               | 3.70±0.21    | 30.60         | 8.38±0.31     | 69.31       | 12.09±0.38a | 10.06±0.97  | 30.58±0.87  |
| T2               | 3.45±0.23    | 28.09         | 8.82±0.40     | 71.82       | 12.28±0.18a | 9.82±0.31   | 31.05±0.38  |
| T3               | 3.83±0.01    | 32.99         | 7.79±0.38     | 67.09       | 11.61±0.39a | 10.10±1.03a | 28.87±1.36a |
Cd alone had less drastic effects than the combined application of Cd and salt. Similarly, Cd and salt imposed stresses in wheat which significantly reduced shoot and root dry weight in wheat genotypes (Shafi et al., 2011). Jampasri (2015) also reported that salt stress (7.79 dS/m) and contaminants (780 mg/kg Pb, 27,000 mg/kg fuel oil) affected the biomass and shoot height of Chromolaena odorata after 90 days of treatment. Despite the fact that Cd is very toxic to plants and the Cd-tolerant threshold in most reported hyperaccumulators were below 25 mg/kg of soil (Wei et al., 2004). Moreover, most crop plants have been assessed as sensitive to ECe values > 4 dS/m (Munns, 2005). However, we found that the two tested species of grass were highly tolerant to Cd and salt with little adverse effect on growth at 20, 60 and 100 mg/kg Cd with an ECe value of 4.87 dS/m (1,000 mg/kg NaCl). Our results showed that V. nemoralis was comparatively less influenced by Cd and salt as compared to V. zizanioides. Similar results were observed by Jampasri et al. (2017) who established that V. nemoralis could tolerate up to 333 mg/kg of soil Cd concentration under salt stress (ECe 3.5 dS/m). However, Liu et al. (2016) and Truong (1994) also showed that V. zizanioides had a salinity threshold of 10.0 and 8.0 dS/m, respectively, without Cd contamination. Generally, drought causes the increase in salt content in the farmland, and also the effect of drought or soil water stress decreased plant’s ability to deal with heavy metals soil contamination. Results from previous study found that the upland switch grass showed a lesser response to drought stress, and exhibited a higher leaf percentage of total dry matter than the lowland cultivars (Stroup et al., 2003). Thus, it is highly possible that upland vetiver grass species (V. nemoralis) has been widely recognized to be more drought resistant with Cd contamination than lowland species. While using lowland grass to remove heavy metals may be required the use of a complex variety of biologi-
cal and phyciochemical processes.

**Accumulation of Cd in plants**

Cd accumulations in roots and shoots increased with increasing Cd concentration (Table 4). The root was the main part of plants to accumulate Cd with and without the addition of salt in the soil. The results showed that salinity did not affect Cd accumulation in roots, while Cd accumulation in shoots was affected by salinity exposure (Figs. 1 and 2). However, V. zizanioides showed a markedly lower

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**Table 4** Cd accumulation (mg/kg DW) and BCF of V. nemoralis (Ratchaburi ecotypes) and V. zizanioides (Songkhla 3 ecotypes) in soil for 60 days. The values consisted of the mean±SE (n = 3). The identical superscripts on the bars indicated no significant difference (P > 0.05) between means and according to the LSD test.

| Treatment | C1 | C2 | C3 | T1 | T2 | T3 | BCF Root | BCF Shoot | TF Root | TF Shoot |
|-----------|----|----|----|----|----|----|---------|-----------|---------|----------|
| V. nemoralis (Ratchaburi ecotypes) | 10.11±0.012 | 9.94±0.016 | 3.28±0.001 | 11.32±1.000 | 10.51±0.000 | 4.58±0.000 | 0.72±0.001 | 0.54±0.000 | 0.68±0.000 | 0.31±0.000 |
| V. zizanioides (Songkhla 3 ecotypes) | 123.58±0.16 | 29.98±0.16 | 274.75±0.22 | 289.20±0.68 | 25.32±1.85 | 212.87±0.53 | 154.88±0.29 | 1.55±0.003 | 1.22±0.000 |
root Cd accumulation than *V. nemoralis* at various Cd concentrations. The highest Cd accumulation in roots (861.94 mg/kg) was recorded for the T3 of *V. nemoralis* (100 mg/kg Cd, saline soil). Both salt and Cd stresses (T1–T3 treatments) decreased shoot Cd accumulation in both grass species. The BCF values in all treatments were higher than 1 except the BCF shoot values in the T1 and C1 of *V. nemoralis* with 20 mg/kg Cd. *V. nemoralis* had higher BCF root values (8.05–11.32) than *V. zizanioides* (6.18–9.12). However, no significant differences in the BCF of roots and shoots were observed among these treatments. The TF values were all less than 1 in Cd treatments and C and salt treatments. The TF values did not significantly (P > 0.05) change among various Cd treatments (C1–C3) and Cd with the addition of salt of both grass species. These results suggested that Cd mainly accumulated in roots and less Cd was transported to shoots in *Vetiveria* spp., while *V. zizanioides* accumulated less Cd in total compared to *V. nemoralis*.

The root Cd accumulation of *Vetiveria* spp. increased with increasing Cd concentration and it was possible to confer high Cd tolerance in these plants. It has also been reported that plant genotypes differ in their tolerance to Cd toxicity (Gill et al., 2011). The results showed that salt also caused an increase in the availability of Cd, as indicated by a significant increase in root Cd accumulation. In addition, saline soils possess high concentrations of potassium ions, chloride ions, and sulfate ions. An increased salinity, which is a cation, may contribute to cation displacement of heavy metal stored in the sediment (Ouadjenia-Marouf et al., 2010). This observation confirmed earlier findings that increasing the levels of NaCl addition in the soil increased Cd availability. Evidently, increasing salinity increased root Cd accumulation in saltcedar (*Tamarix Smyrnensis*) and saltbush (*Atriplex halimus*) (Lutts et al., 2004; Manoussaki et al., 2008). Smolders et al. (1998) reported that the increase of salinity promoted Cd availability in Swiss chard (*Beta vulgaris*). Similarly, the increase of root Cd accumulation in saline soils was also reported in many crop plants (Norvell et al., 2000; Zhao et al., 2013).

In the present study, both vetiver species were not suitable for phytoextraction due to their comparatively high BCF but low TF values with increasing Cd concentrations under salinity. Although many researchers reported high salinity which could enhance the heavy metal translocation from roots to shoots of halophyte species (Ghinya et al., 2007; Han et al., 2012b; Mariem et al., 2014). However, *Vetiveria* spp. are not halophytes and the results contrasted with the previous studies because salinity could increase Cd accumulation in the aboveground parts of the plants (Gabriel et al., 2009). Vetiver grasses generally accumulate more Cd in roots. This might be a protective mechanism for plants to accumulate heavy metals in their roots and vacuoles and limit transportation to plant shoots (Pandey et al., 2014). Cd accumulation in shoots directly leads to damage to the photosynthetic apparatus (Dias et al., 2013). On the other hand, the high Cd accumulation in roots is probably due to Cd absorption into the negatively charged surface of the root cell walls (Solis-Dominguez et al., 2007). This low Cd translocation is not only a characteristic but could be the main cause of the higher resistance of vetiver grass to Cd. Consequently, vetiver grass could be regarded as a candidate species for the phytostabilization of Cd contamination, which not only beautified the environment but also reduced the risk of food chain pollution (McIntyre, 2003). In general, the candidate plants for phytostabilization should have an extensive root system to accumulate high amounts of metals (Alvarenga et al., 2008).

This study demonstrated higher root Cd accumulation in *V. nemoralis* than *V. zizanioides* exposed to Cd and salt stresses. *V. nemoralis* exhibited the highest root Cd accumulation at 100 mg/kg Cd, with the addition of salt in the soil. Roongtanakiat and Chairoj (2002) also reported that there was a significantly higher concentration of Cd in *V. nemoralis*, compared with the other *Vetiveria* spp. in the pot experiment with or without addition of salt. Under the combined stress, the results demonstrated that both tested vetiver grass ecotypes could effectively act as phytostabilizer for Cd in which most Cd was mainly retained in the roots. They were fast growing and had a greater biomass when compared to other species of grass. Despite the fact that they are not hyperaccumulators but can still be considered a good candidate to stabilize Cd in polluted soil and/or in coastal ecosystems.

**CONCLUSIONS**

The vetiver grasses, *V. zizanioides* and *V. nemoralis*, could grow in contaminated soil with varying levels of Cd, with only a slight reduction in dry biomass, root length and shoot height. Salt stress can inhibit and limit plant growth and Cd translocation of *Vetiveria* spp. Among the two tested species, *V. nemoralis* had more accumulation ability for higher concentrations of Cd than *V. zizanioides*. The present study also revealed the bioaccumulation ability of roots which was found to be higher than that of shoots after 60 days of Cd exposure. When grown in contaminated saline soil with moderate and high Cd concentrations, two vetiver grass species had the capacity to immobilize Cd from roots to shoots. Hence, they are suitable for Cd phytoextraction and unsuitable for Cd phytoextraction with the salinity level of 1,000 mg/kg. Of the two species tested, *V. nemoralis* may represent a good possible interventional for the phytoremediation of Cd-contaminated soils with these conditions.

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