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In situ stabilization remediation of cadmium contaminated soils of wastewater irrigation region using sepiolite

Yuebing Sun¹,², Guohong Sun³, Yingming Xu¹,*, Lin Wang¹,², Dasong Lin¹,², Xuefeng Liang¹,², Xin Shi⁴

1. Key Laboratory of Original Environmental Quality Safety, Ministry of Agriculture, Tianjin 300191, China. E-mail: sunyuebing2008@yahoo.com.cn
2. Tianjin Key Laboratory of Agro-environment and Agro-product Safety, Institute of Agro-Environmental Protection, Ministry of Agriculture, Tianjin 300191, China
3. Department of Basic Science, Tianjin Agricultural University, Tianjin 300384, China
4. College of Environment and Resources, Jilin University, Changchun 130012, China

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Abstract

The effects of immobilization remediation of Cd-contaminated soils using sepiolite on soil pH, enzyme activities and microbial communities, TCLP-Cd (toxicity characteristic leaching procedure-Cd) concentration, and spinach (Spinacia oleracea) growth and Cd uptake and accumulation were investigated. Results showed that the addition of sepiolite could increase soil pH, while the TCLP-Cd concentration in soil was decreased with increasing sepiolite. The changes of soil enzyme activities and bacteria number indicated that a certain metabolic recovery occurred after the sepiolite treatments, and spinach shoot biomass increased by 58.5%–65.5% in comparison with the control group when the concentration of sepiolite was 610 g/kg. However, the Cd concentrations in the shoots and roots of spinach decreased with an increase in the rate of sepiolite, experiencing 38.4%–59.1% and 12.6%–43.6% reduction, respectively, in contrast to the control. The results indicated that sepiolite has the potential for success on a field scale in reducing Cd entry into the food chain.

Key words: Cd-contaminated soil; immobilization remediation; sepiolite; soil quality

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Introduction

Cadmium (Cd) is one of the most toxic pollutants in the soil environment because of its persistence, toxicity and potential for bioaccumulation (Bebianno et al., 1994; Zhou and Song, 2004). Cd mainly affects the kidneys, especially the proximal tubular cells. It also contributes to many mental illnesses, particularly violence and other related disorders of behavior and mental attitude, and even causes "itai-itai disease" (Horiguchi et al., 1994; Sun et al., 2009). Therefore, Cd is banned by European Union’s Restriction of Hazardous Substances Directive from 2006 (European Union, 2002).

The spreading of untreated sewage effluent on land, especially in arid and semiarid regions, is considered as a practical and attractive alternative because of the increasing scarcity and high cost of freshwater resources (Sun et al., 2009). In China, urban wastewater has been considered as one of the most important freshwater resources and has been used for agricultural irrigation since the 1950s (Sun et al., 2009, 2011). The area of wastewater irrigation has increased from 4000 km² in 1978 to 30,000 km² in 2003, and 64.8% of the area has suffered some degree of heavy metal contamination (Zhou and Song, 2004). However, as a side-effect of long-term application of untreated wastewaters, trace elements can also accumulate in the soil at toxic levels. Excessive accumulation of heavy metals in agricultural soils has led to elevated heavy metal uptake by crops, and thus affects food quality and safety (Wang et al., 2010b). It is estimated that there are about 12 million tons of grain crops contaminated with heavy metals every year in China, which causes direct economic losses of more than 20 billion CNY (Chen, 1996). The arable land contaminated by Cd is 13,000 ha, involving 25 regions in 11 provinces, where the quantity of Cd-contaminated rice reached 50 million kg in 1999 (Wang et al., 2010a; Li et al., 2009). Consequently, the high concentration of Cd in rice has posed hazardous effects to human health by way of the food chain and Cd contamination events have emerged with a high frequency recently (Sun et al., 2011). Therefore, the cleanup of Cd-contaminated soils is imperative.

Numerous remedial technologies have been proposed and investigated for treating heavy-metal-polluted soils including chemical stabilization techniques, which aim to reduce metal mobility and bioavailability in soils by ad
1.1 Natural sepiolite properties

The natural sepiolite sample was purchased from Yixian Dazhi Heat-Rreservation Construction Materials Co., Ltd., China. Brunauer-Emmett-Teller measurements revealed that the sepiolite had a surface area of 22.70 m²/g, and the average pore size was determined as 1.4 nm.

1.2 Soil characterization and plant culture

The soil sample (0–20 cm) was collected from agricultural fields in the Zhangshi Irrigation Region in Shenyang, China (Table 1). The soil was ground to pass through a 4 mm mesh and placed in plastic pots. Then the sepiolite was blended into the contaminated soil at the concentration of 0, 5, 10, 15, and 25 g/kg, respectively. Each treatment was replicated in triplicate and the soil was incubated for 5 weeks. Six seeds of spinach were planted into each pot. Loss of water was made up using tapwater (no Cd detected) to reach 75% of the field water-holding capacity prior to analysis.

1.3 Analytical methods

1.3.1 Soil pH and Cd speciation

The pH was measured at a soil:water ratio of 1:2.5 (V/V) using a pH meter (PB-10, Sartorius, Germany). The toxicity characteristic leaching procedure (TCLP) followed USEPA Method 1311 (USEPA, 1986). Two acetic-acid-based TCLP extraction fluids were used: extraction fluid #1 (pH 4.93 ± 0.05) and extraction fluid #2 (pH 2.88 ± 0.05). Fluid #1 was applied when the pH of the slurried sample was less than 5. Fluid #2 was applied when the slurry pH was above 5 and remained so after the addition of 1 mol/L HCl. However, when the pH dropped below 5 upon HCl addition, fluid #1 was used. Therefore, both soil pH and buffering capacity were considered when making the extraction fluid selection. After 18 hr of mixing in a TCLP tumbler, the leachate was filtered using a Millipore 0.4 μm filter. The pH of the filtrate was then measured and the leachate was acidified with a small amount of nitric acid to a pH less than 2, followed by atomic adsorption spectrometry analysis (Dermatas et al., 2006).

1.3.2 Estimation of numbers of soil microorganisms

The number of soil microorganisms was estimated by the dilution plate technique (three replicates from each dilution and soil dish). The compositions and preparation of media were as follows (Shen et al., 2005): (1) Soil bacteria: beef extract 5 g, peptone 10 g, NaCl 5 g, distilled water 1000 mL, agar 18 g, pH 7.2–7.4. The bacterium-mixing plate method was used and 10⁻⁵–10⁻⁷ dilutions of soil sample were used as inoculums. The colonies were counted after incubation at 30°C for 3 days. (2) Fungi: potato without peel 200 g, sucrose 20 g, distilled water 1000 mL, agar 18 g, pH natural. The potato without peel was cut into small pieces and boiled in 1000 mL distilled water for 25–30 min, then filtered with double layers of cheesecloth. The sucrose and distilled water were added into the filtrate up to 1000 mL. The 10⁻¹–10⁻³ dilutions of soil sample were used as inoculums. The colonies were counted after incubation at 30°C for 3 days. (3) Actinomycetes: soluble starch 10 g, (NH₄)₂SO₄ 2 g, K₂HPO₄ 1 g, MgSO₄·7H₂O 1 g, NaCl 7 g, CaCO₃ 3 g, distilled water 1000 mL, agar 18 g, pH 7.2–7.4. 10⁻³–10⁻⁵ dilutions of soil sample were used as inoculums. The colonies were counted after incubation at 30°C for 5 days.

1.3.3 Soil enzyme activities

Soil urease activity was determined by the method of Tabatabai (1994). Urease activity was determined with urea as substrate, incubating at pH 7.1 (0.2 mol/L phosphate buffer) and 37°C for 24 hr. The residual urea was determined by a colorimetric method. The enzymatic activity is expressed as mg NH₄⁺-N/(g·hr).

The catalase activity was analyzed by titration with KMnO₄. Soil samples were added to 40 mL distilled water with 5 mL of 0.3% H₂O₂. The mixture was shaken for...
20 min and then 5 mL of 1.5 mol/L H$_2$SO$_4$ were added. Afterwards the solution was filtered and titrated using 0.1 mol/L KMnO$_4$. The reacted amount of 0.1 mol/L KMnO$_4$, calculated per gram of dry soil, was used to express the activity of catalase. The enzymatic activity is expressed as mL/(g·hr) (Slipe et al., 2009).

Invertase activity was determined using a sucrose solution as a substrate and incubation at 37°C for 24 hr, before measuring the produced glucose with a colorimetric method. The enzymatic activity is expressed as mg/(g·hr) (Kandeler et al., 1999).

### 1.3.4 Cd determination

The plant and soil samples were digested with a solution of HNO$_3$-HClO$_4$ and HCl-HNO$_3$-HF-HClO$_4$, respectively. The concentrations of Cd were determined using atomic absorption spectrophotometry (Solaar M6, Thermo Fisher Scientific, USA). A certified reference material, bush leaf material (GBW07603, China), was used to verify the accuracy and precision of the digestion procedure and subsequent analysis.

The bioaccumulation factor (BF) is defined as the ratio of metal concentration in the plants to metal concentration in the soil, and the transfer factor (TF) is defined as the ratio of the concentration in the stems to that in the roots (Sun et al., 2008).

### 1.4 Statistical analysis

All treatments were replicated three times in the experiments. The means and standard deviations (SD) were calculated by Microsoft Office Excel 2003. One-way analysis of variance was carried out with SPSS10.0. When a significant ($P < 0.05$ or $P < 0.01$) difference was observed between treatments, multiple comparisons were made by the LSD test.

### 2 Results and discussion

#### 2.1 Cd contamination in the Zhangshi Irrigation Region

The soil used in this study was slightly acidic and showed a Cd level of (5.12 ± 0.19) mg/kg dry soil (Table 1). According to the environmental quality standards for heavy metals in soils (GB 15618-1995), the concentration of Cd was over 5.1 times higher than the corresponding heavy metals in soils (GB 15618-1995), indicating that the soils were seriously polluted by Cd. The result was consistent with the report by Wu et al. (1989) who found that the Cd concentrations at Sluice Gate I in the Zhangshi Irrigation Region ranged from 5 to 7 mg/kg, and 3–5 mg/kg at Sluice Gates II and III. High Cd content in soils has posed a potential hazard to human health through the food chain. Wu et al. (1989) found that the Cd concentration in human blood in the same region also increased from 1.76 µg/L in 1978 to 2.23 µg/L in 1979, and then up to 13.26 µg/L in 1982. A survey of renal function in adult women in this area was carried out by Yuan et al. (1992), in which the positive excretion rate of low molecular weight protein and the activity of urinary β2-MG and ALP in urine of the investigated women at the Cd-polluted sites were significantly higher than those in the control, indicating that renal dysfunction had appeared in some women. Hence, the remediation of Cd-contaminated soils in the Zhangshi Irrigation Region is imperative.

#### 2.2 Effect of amendment on soil pH and Cd speciation

As shown in Table 2, before planting spinach, the application of sepiolite significantly increased the soil pH, which increased with the increasing amount of sepiolite applied to the soil. This is due to the sepiolite containing a significant percentage of calcium carbonate (CaCO$_3$), giving it alkaline properties (pH 10.1). A similar pattern in pH variation was observed in the soils after harvesting the plants, which showed that the addition of sepiolite to the soils caused a slow increase in pH. Only the treatment of 25 g/kg sepiolite significantly enhanced soil pH when compared with the control ($P < 0.05$).

The stability of metals in soil is strongly pH dependent. The mobility and availability of heavy metals increase with declining soil pH value (Kumpiene et al., 2008). It has been deduced that most metals are chemically immobilized due to surface complexation as pH is increased (Querol et al., 2006). As shown in Table 2, the highest Cd concentration in TCLP leachate (0.67 mg/kg) was obtained in the unamended soil sample at the rate of 13.0%. After applying sepiolite to soil, the TCLP-Cd concentrations decreased with the increase of sepiolite, resulting in between 20%–25.4% reduction compared with the control (significant decreases at all levels of sepiolite addition, $P < 0.05$). Similar trends were found by Wang et al. (2010) for TCLP-extractable heavy metals under the treatments of natural clay minerals such as kaolinite and bentonite.

### Table 2 Response of pH, Cd available concentration and microbial properties under different treatments

<table>
<thead>
<tr>
<th>Sepiolite (g/kg)</th>
<th>pH Before planting</th>
<th>pH After harvesting</th>
<th>TCLP for Cd</th>
<th>Bacteria ($\times 10^7$ CFU/g soil)</th>
<th>Fungi ($\times 10^5$ CFU/g soil)</th>
<th>Actinomycete ($\times 10^5$ CFU/g soil)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>6.69 ± 0.01 d</td>
<td>7.46 ± 0.09 b</td>
<td>0.67 ± 0.00 a</td>
<td>13.0</td>
<td>1.58 a</td>
<td>2.10 b</td>
</tr>
<tr>
<td>5</td>
<td>7.13 ± 0.08 c</td>
<td>7.47 ± 0.11 b</td>
<td>0.56 ± 0.05 b</td>
<td>10.4</td>
<td>1.19 b</td>
<td>2.15 b</td>
</tr>
<tr>
<td>10</td>
<td>7.38 ± 0.01 b</td>
<td>7.51 ± 0.10 b</td>
<td>0.53 ± 0.02 b</td>
<td>11.0</td>
<td>1.47 b</td>
<td>2.20 b</td>
</tr>
<tr>
<td>15</td>
<td>7.48 ± 0.11 ab</td>
<td>7.60 ± 0.04 ab</td>
<td>0.50 ± 0.04 b</td>
<td>9.7</td>
<td>1.22 b</td>
<td>2.70 a</td>
</tr>
<tr>
<td>25</td>
<td>7.64 ± 0.11 a</td>
<td>7.73 ± 0.00 a</td>
<td>0.51 ± 0.04 b</td>
<td>9.9</td>
<td>1.27 b</td>
<td>2.40 ab</td>
</tr>
</tbody>
</table>

TCLP: toxicity characteristic leaching procedure.
2.3 Plant growth and Cd concentration

The biomass dry weight of shoots usually reflects the tolerance capability of plants to adverse environments (Wei and Zhou, 2004; Sun et al., 2009). Stabilization of contaminants in soil can be achieved by amendments able to adsorb, complex or (co)precipitate trace elements, and thus reduce metal phytotoxicity to crops by decreasing their mobility and bioavailability (Bolan et al., 2003; Kumpiene et al., 2008). When the concentration of sepiolite was ≤ 10 g/kg, the application of sepiolite enhanced plant growth and production, resulting in 58.5%–65.5% increase in comparison with the control, although there was no statistical differences between the treatments (P > 0.05) (Fig. 1). The results above indicated that a moderate concentration of sepiolite could alleviate the stress of Cd to the plant. In contrast, the addition of sepiolite had an inhibitive effect on plant growth when it was increased to 15 g/kg and above. Shoot biomass significantly decreased in contrast to that of the control (P < 0.05), by 21.1% and 15.3%, for the sepiolite levels of 15 and 25 g/kg, respectively.

Numerous research has shown that spinach has a high capacity to absorb Cd (Chunillall et al., 2004; Ackzai and Bazai, 2006; Chiroma et al., 2007). The concentration of Cd in spinach significantly and linearly increased with the level of Cd in wastewater at Chiltan Gbee Mill, Chiltan Town and Zarghoon Town in Quetta City, Pakistan, and the maximum amount of Cd reached 11.2, 6.1 and 7.4 mg/kg, respectively (Ackzai and Bazai, 2006). Chunillall et al. (2004) found the Cd content in spinach plants grown on 10 mg/kg Cd-contaminated soil after a 35- and 70-day exposure was 22.0 and 26.14 mg/kg, respectively. In this study, the concentrations and distribution of Cd in spinach are shown in Fig. 2. When no sepiolite was applied, the Cd concentration in shoots and roots of spinach reached 7.4 and 20.9 mg/kg, respectively (Fig. 2a). The high concentration of Cd accumulated in spinach could cause health risks through the food diet.

Immobilization materials such as phosphorus-containing amendments (Ma et al., 1995; Fayiga and Ma, 2006), alkaline compounds (Lombi et al., 2002; Brown et al., 2004), clay minerals (Xu et al., 2003; Liang et al., 2011; Li et al., 2009), and biosolids (Brown et al., 2004; Kumpiene et al., 2008) have been proven to be very effective in reducing the bioavailability of metals in soils, thus hampering their plant absorption and translocation (Zhou and Song, 2004). After application of zeolite, the Pb concentration of the aerial parts of rape was restrained by 47% at low Pb concentration (≤ 500 mg/kg) treatment level, and 30% at high Pb concentration (> 1000 mg/kg) treatment level (Li et al., 2009). In the present study, the sepiolite amendment employed was very efficient at reducing Cd concentration by the plants (Fig. 2a). After application of sepiolite (5–25 g/kg), the Cd contents in the shoots of spinach were dramatically decreased (P < 0.05), resulting in 38.4%–59.1% reduction relative to the no sepiolite treatment. Similarly, the root Cd concentration was gradually inhibited with increasing soil sepiolite concentration, with a decrease of 56.4%–87.4% of the control value, and significantly decreased when the concentration of sepiolite was increased to 10 g/kg (P < 0.05). Therefore, sepiolite could be used as an effective alternative material for immobilization remediation of Cd-contaminated sites.

2.4 Soil enzyme and microbial properties

Soil enzyme activities are widely used as biological indicators for soil quality due to their intimate relationship with soil biology, ease of measurement, and rapid response to change in soil management (Dick, 1997; García-Ruiz et al., 2009). Soil enzymes are also used to estimate the adverse effects of various pollutants on soil health (Hinojosa et al., 2008). Catalase is a kind of oxidoreductase which can accelerate the degradation of hydrogen peroxide and protect organisms from the toxicity of hydrogen peroxide (Cang et al., 2009), and has been used as an indicator of soil fertility (Trasar-Cepeda et al., 2007). Urease catalyzes the hydrolysis of urea into ammonia or ammonium ion, it is important to uncover other unknown factors that may reduce the efficiency of this enzyme in the ecosystem. (Gu...
Invertase catalyses the hydrolysis of sucrose into glucose and fructose, and is more efficient than other enzymes in reflecting soil fertility and biological activity level (Cang et al., 2009).

Efficient in situ immobilization of heavy metals may benefit soil functionality by decreasing labile heavy metals (de Mora et al., 2005), and the recovery of contaminated soil should be assayed not only by soil chemical properties determined by conventional analytical tests and extraction procedures, but also by evaluation of the restoration of soil habitat function (Lee et al., 2009). As shown in Fig. 3, the catalase activity increased gradually with the various treatments of sepiolite, although it did not rise dramatically until sepiolite addition was increased to 15 g/kg. Compared with the control, catalase activity increased by between 3.0%–26.3% after applying sepiolite to the soil. The activities of urease and invertase first increased and then decreased with increasing sepiolite concentration, and reached a maximum of 1.62 mg NH$_4^+$-N/(g·hr) and 2.15 mg/(g·hr), respectively, at the sepiolite additions of 5 and 10 g/kg, respectively. Urease activity increased between 0.3% and 69.8% at sepiolite additions of 5–15 g/kg when compared with control, while invertase activities decreased by 17.7%–22.7% relative to the control under sepiolite additions of between 10–25 g/kg. Heavy metals may complex the substrate, combine with the protein-active groups of the enzymes, or react with the enzyme-substrate complex, and metals can react with sulphydray groups causing inactivation or inhibition of enzyme activity (Yang et al., 2006). However, the physical and chemical properties of the soil, such as organic matter concentration, type and amount of clay mineral and soil pH, influence the toxic effects of trace elements (Sinha et al., 1978; Yang et al., 2006). These processes are perhaps responsible for the variation in the inhibition or activation of soil enzyme activities observed in the soil with different sepiolite levels, and thus a moderate content of sepiolite can alleviate the toxic effects of Cd to the activities of catalase, urease and invertase.

The quantitative analysis of the microbe population is shown in Table 2. The amount of bacteria under the sepiolite treatments was inhibited, being about 7.0%–22.8% lower than that of the control. In contrast, the application of sepiolite increased the number of fungi and actinomycetes, resulting in 2.4%–28.6% and 0.3%–7.5% increase, respectively, with regard to the control. The trends of fungi and actinomycete amounts increased first, and then decreased with increasing sepiolite content. However, only the 15 g/kg sepiolite addition significantly increased the amount of fungi, and, there were no significantly differences in soil actinomycete counts between the various treatments. Bacteria, fungi and actinomycetes play an important role in the formation of soil aggregates in good soil structure (Zhou and Song, 2004); they promote the detoxification of soil contaminants and the production of plant growth promoters and organic chelating agents. However, since trace elements exhibit toxic effects towards soil biota: they can affect key microbial processes (Obbard, 2001), and decrease the amount and activities of soil microorganisms (de Mora et al., 2005). The positive effect of sepiolite on fungi and actinomycete count (Table 1) may be attributed to the effects of amendment on decreasing metal toxicity (Lee et al., 2004), showing the restoration of Cd-contaminated ecosystems after applying sepiolite (de Mora et al., 2005).

### 2.5 Potential of using sepiolite for stabilization remediation of Cd-contaminated soil

The values of BF and TF are usually calculated to evaluate the effectiveness of metal accumulation and translocation (Zhou and Song, 2004; Sun et al., 2009, 2011). When growing in unamended soils, the values of BF in shoots and roots of spinach were > 1.0 (Fig. 2b), indicating spinach has a high ability for Cd uptake and accumulation. However, the addition of sepiolite apparently inhibited Cd uptake and translocation: the BF in the roots and shoots reduced by 10.5%–37.6% and 37.0%–56.9%, respectively, compared with the control when 5–25 g/kg sepiolite was added to the soil, and TF values decreased by 8.9%–37.0% relative to that of the control.

The concentration of Cd in the shoots of spinach after applying sepiolite to soil was significantly reduced ($P < 0.05$), and decreased with the increase of soil sepiolite (Fig. 4). The Cd contents in the edible parts of the plants under the treatments of 5–25 g/kg sepiolite decreased by 32.8%–58.6% in comparison to the control. Wang et al. (2010a) reported that the treatments of 6% and 9% sepiolite dramatically decreased the Cd concentration in roots and shoots of Brassica chinensis, decreasing it by 26.4% and 27.9% for shoots, and 11.1% and 13.4%
for roots, respectively. Under the combined treatment of sepiolite and phosphate fertilizer, the Cd concentrations in shoots and roots of B. chinensis experienced a reduction of 59.8% and 27.6%, respectively, when compared with that of the control (Wang et al., 2010a). According to Liang et al. (2011), the Cd and Pb concentrations after applying natural clay and phosphate fertilizer decreased by 51.8% and 45.1% in Lactuca sativa L., 47.0% and 25.2% in Brassica campestris L., and 24.9% and 41.9% in Raphanus sativus, respectively. Therefore, the application of sepiolite and its compound material should be applied for immobilization remediation of Cd-contaminated soil to ensure the safety of food to humans.

3 Conclusions

The experimental results indicated that sepiolite-induced stabilization is an effective technique for remediation of Cd-contaminated soils. The addition of sepiolite led to a 0.1%–14.1% increase in soil pH and a 0.8%–10.8% decrease in the TCLP of soil Cd. The dry weight of shoots of spinach reached a maximum at the rate of 5 g/kg sepiolite and then suffered a certain degree of decrease when it was increased to 15 g/kg. The Cd concentrations in the shoots and roots of spinach were reduced by 38.4%–59.1% and 12.6%–43.6% in contrast to the control when sepiolite was applied to the soil. The amendment could restore soil functional properties as reflected in the changes in soil enzyme activities and microbial counts. In conclusion, the application of sepiolite amendment that can immobilize Cd in situ may provide a cost-effective and sustainable solution for the remediation of Cd-contaminated soils.

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