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Reliability and stability of immobilization remediation of Cd polluted soils using sepiolite under pot and field trials

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ABSTRACT

Long-term effectiveness and persistence are two important criterias to evaluate alternative remediation technology of heavy metal polluted soils. Pot and field studies showed addition of sepiolite was effective in immobilizing Cd in polluted soils, with significant reduction in TCLP extracts (0.6%–49.6% and 4.0%–32.5% reduction in pot and field experiments, respectively) and plant uptake (14.4%–84.1% and 22.8%–61.4% declines in pot and field studies, correspondingly). However, the applications of sepiolite offered a limited guarantee for the safety of edible vegetables in Cd-polluted soils, depending on the soil type, the Cd pollution type and level, and the dose and application frequency of chemical amendments. Bioassays, such as plant growth, soil enzymatic activities and microbial community diversity, indicated a certain degree of recovery of soil metabolic function. Therefore, sepiolite-assisted *in situ* remediation is cost-effective, environmentally friendly, and technically applicable, and can be successfully used to reduce Cd enter into the food chain on field scale.

1. Introduction

Cadmium (Cd) pollution in agricultural soils has steadily increased in the last few decades because of increases in metal mining and smelting operations (Ettler et al., 2012; Yu et al., 2006), irrigation with industrial wastewater (Álvarez-Ayuso and García-Sánchez, 2007; Chary et al., 2008; Sun et al., 2009), uses of chemical fertilizers and pesticides (Hseu et al., 2010; Huang et al., 2007), and so on. A nationwide survey carried out by the China Ministry of Environmental Protection and the China Ministry of Land and Resources from 2005 to 2013 has revealed that Cd was the most frequently detected heavy metal in soils, and that 7% of the investigated sites were polluted by Cd according to the Chinese Environmental Quality Standard for Soils (http://english.mep.gov.cn/News_service/news_release/201404/t20140428_271088.htm). There was about 1.3×10^7 hm^2 of agricultural soils polluted by Cd, covering 25 regions of 11 provinces (Gu et al., 2003). Excessive accumulation of Cd in soils has led to elevate Cd uptake by crops and inevitably poses risk to human health via food chain. The surveillances of renal function state in adult women revealed that the positive rate of low molecular weight protein and the urinary β2-MG and ALP activities in the urine of women in Cd polluted area were significantly higher than those of control group, indicating that the renal dys (EFSA, 2009) function had appeared in the part of women (Xu et al., 2003). Moreover, newer data on human exposure to Cd in the general population has showed that increased risk of cancer, such as in the lung, endometrium, bladder, and breast, is statistically associated with Cd pollution (EFSA, 2009). So, remediation and cleanup of Cd-polluted soils is imperative and emergent.

Usually, there are two types of remediation strategies used for remediation and cleanup of heavy metal polluted soils: *in situ* enhancement of immobilization of metals on soil particles (e.g., stabilization by chemical amendments and phytostabilization) (Cao et al., 2002; Mendez and Maier, 2008; Sun et al., 2013; Zanuzzi et al., 2013), and *ex situ* extraction or separation of metals from polluted soils (e.g., phytoextraction, leaching and flotation) (Coruh et al., 2013; Shim et al., 2014; Wuana and Okieimen, 2011).

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Agriculture-based *in situ* chemical immobilization is applications of inexpensive materials, such as alkaline materials (Illera et al., 2004), Al/Fe/P-containing compounds (Michálková et al., 2014; Udeigwe et al., 2011), clay minerals (Liang et al., 2014; Sun et al., 2013), and organic amendments (Liu et al., 2009; Park et al., 2011). These materials minimize the bioavailability and transport of heavy metals in soil environment by increasing soil pH or enhancing adsorption, ionic exchange, complexation or precipitation reactions (Cao et al., 2008; Sun et al., 2009; Udeigwe et al., 2011). Chemical immobilization has recently been gaining prominence because of its cost-effectiveness, rapid implementation and environmental sustainability. It is also considered as a potentially valuable alternative techniques for vast industrial sites and agriculture lands, dumping grounds or soils that have been highly polluted (Illera et al., 2004; Zanuzzi et al., 2013).

A thorough evaluation of the effectiveness of chemical materials for immobilization of heavy metals in soil remediation practices involves not only the assessment of soil chemical characteristics by chemical and physical approaches (Cheng and Hseu, 2002), but also the analyses of restoration of soil habitat function by biological methods (Adriano et al., 2004). The soluble and exchangeable fractions of trace elements are the most important pools of toxicity and bioavailability in soils (Usman et al., 2005). The plant-based bioassay (biomass and heavy metal content) is an end-point measure to evaluate the phytotoxicity of soil heavy metals (Sun et al., 2008). Soil biological indices (soil enzymatic activities and microbial communities) can directly address biological availability and toxicity of heavy metals, and help define the acceptable cleanup standards. These indices have often been proposed as sensitive and early indicators of soil ecological stresses caused by restoration processes (Calbrix et al., 2007; Kumpiene et al., 2006).

A successful immobilization remediation technique must maintain reasonable low solubility and bioavailability of heavy metals and should also improve soil ecological function with the universality and long-term (Basta et al., 2001; Ruttens et al., 2010). However, most studies have been carried out in laboratory and/or small-scale applications. These results provide little guarantee for permanently safe crop production at a large-scale or in long term (Basta et al., 2001; Sun et al., 2013).

In order to evaluate the reliability and stability of immobilization remediation of Cd polluted soils, we investigated the potential of sepiolite for immobilization remediation of Cd polluted soils using pot experiments (three artificially and three naturally polluted soils) and a three-year field experiment. The mobility and bioavailability of soil Cd was investigated using the toxicity characteristic leaching procedure (TCLP). Soil microbial population and enzymatic activities and plant Cd concentrations were also conducted to assess the effectiveness of Cd immobilization remediation using sepiolite.

2. Materials and methods

2.1. Sepiolite and soil properties

The natural sepiolite [$Mg_4Si_6O_{15}(OH)_2 \cdot 6H_2O$] was purchased from Hebei Sepiolite Developing Co., LTD. It had a surface area of 22.70 m^2 g^{-1} determined by the Brunauer–Emmett–Teller (BET) method and a mean pore size of 1.4 nm measured by the Barrett–Joyner–Halenda (BJH) method.

Three naturally polluted soil samples (0–20 cm) were collected from Hechi (HC), Shenyang (SY) and Tianjin (TJ), China. Three unpolluted soil samples (0–20 cm), e.g., red soil (RS), brown soil (BS) and phaiozem soil (PS) were respectively collected from Hunan, Tianjin and Jilin, China. The physical and chemical characteristics of tested soil were analyzed according to Bao (2000). The basic physiochemical properties of the tested soils were listed in Table 1.

2.2. Pot and field plot experimental designs

A 1.0 kg soil sample (HC, TJ, SY, RS, BS and PS) was ground to pass through a 4 mm mesh sieve and placed into a plastic pot. RS, BS and PS soils were mixed with 1.25 mg kg^{-1} Cd as simulated low Cd pollution, according to the Chinese Environmental Quality Standard for Soils (China, 1995). Sepiolite was blended into the artificially and naturally polluted soils at a rate of 0%, 1%, and 5%, and then all pots were incubated for 5 weeks. 6 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 and maintained this humidity by daily watering throughout the cultivation, and a petri dish was placed under each pot to collect potential leachate during the experiment. After 72 days of growth, the plants were harvested. Each treatment was triplicate.

Field experiments were conduced in 2011–2013 in Dongli District, Tianjin, China. The area of experimental plots was 10 m^2 (5 m × 2 m). In the 1st year, sepiolite at 1.1 and 2.3 × 10^4 kg ha^{-1} was added to 18 plots. In the 2nd year, 6 plots treated with two rates of sepiolite were not applied with sepiolite any more, but the rest plots were added successively with 1.1 and 2.3 × 10^4 kg ha^{-1} sepiolite, respectively. In the 3rd year, only 6 plots were treated the 3rd time with same rates of sepiolite. Sepiolite was spread on the surface and then thoroughly mixed by rotary tiller. Plots without sepiolite applications served as control. Each treatment replicated thrice. After 62 days of growth, the vegetable plants were harvested.

The plant samples were washed with tapwater, rinsed 3–4 times with deionized water, and then separated into roots and edible portions (aboveground parts). All samples were oven dried (75 °C) to a constant weight. The fresh weight (FW) and dry weight (DW) of each plant sample were recorded. The plant samples were ground using a stainless steel mill and passed through a 0.25 mm sieve for analyses.

2.3. Analytical methods

2.3.1. Soil pH and available Cd

Soil pH was measured at a soil:water ratio of 1:2.5 using a pH meter (PB-10). Available Cd was determined with the toxicity characteristic leaching procedure (TCLP) described by USEPA Method 1311 (USEPA, 1995).

2.3.2. Soil enzymatic activities and soil microorganisms

Urease activity was assayed with method described by Hu (Hu et al., 2014), expressed as NH_4–N mg g^{-1}. Catalase activity was analyzed by titration with 0.1 mol L^{-1} $KMnO_4$ (Sun et al., 2012), expressed as mL g^{-1}. Invertase activity was determined by incubating soil in sucrose solution at 37 °C for 24 h and measuring the glucose production with a colorimetric method. The enzymatic activity was expressed as mg glucose g^{-1} (Sun et al., 2013).

The population of soil microorganisms (bacteria, fungi and actinomycete) was estimated using dilution plating technique (three replicates for each dilution and soil treatment). Composition and preparation of the media were reported previously (Shen et al., 2005).

2.3.3. PCR-DGGE microbial community analysis

Five grams of soil samples were used for DNA extraction with the FastDNA® Spin Kit for soil (Q-BIO gene, US). The oligonucleotide universal primers used in the amplification for bacteria were UNIV517r (5′-ATT ACC GCG GCT GCT GC-3′) and EUB357f (5′-CCT ACG GGA GGC AGC AG-3′) with a GC-clamp (5′-CGC CCG CCG CGC