Research paper

In situ stabilization remediation of cadmium (Cd) and lead (Pb) co-contaminated paddy soil using bentonite

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ABSTRACT

A pot trial was conducted to evaluate the effectiveness of bentonite in immobilizing Cd and Pb in soils using a set of parameters such as biochemical and physiological characteristics, metal fractions, plant growth and heavy metal concentrations, and soil enzyme activities and microbial community. Results showed that applying bentonite to soil increased activities of superoxide dismutase (SOD) in roots and peroxidase (POD) in leaves and soluble protein (SP) contents in roots of rice seedling by 2.19–2.46, 0.18–0.34, and 0.06–2.20 times, but decreased malondialdehyde (MDA) contents in leaves and roots by up to 74.1% and 62.1%, respectively, as compared with the control. The additions of bentonite reduced the exchangeable fraction of Cd by 11.1%–42.5% and that of Pb by 20.3%–49.3%, most of which was converted into unavailable forms, resulting in 3.0%–54.3% and 6.7%–10.0% increases in residual portions, respectively. Treatments with bentonite inhibited Cd and Pb translocation from soil to the aerial parts of *Oryza sativa* L. Compared with the control, bentonite applications reduced concentrations of Cd and Pb in the roots by 9.4%–31.3% and 5.1%–26.7%, and by 17.4%–44.3% and 3.7%–7.8% in the shoots, respectively. The decreased catalase and increased values of invertase and urease activities and bacteria, fungi, and actinomycete population in the soil implied certain metabolic recovery and soil quality improvements by bentonite additions. These results indicate that bentonite has the potential to reduce heavy metal bioavailability in paddy soils.

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1. Introduction

Intensive industrialization and urbanization, large numbers of manufacturing and agricultural activities, and inadequate waste disposal have resulted in soil contamination by heavy metals (Zhou and Song, 2004; Sun et al., 2009a). Soil heavy metal pollution has become a serious problem in China. According to China's National Soil Pollution Survey Bulletin (NSBSP) issued jointly by the Ministry of Environmental Protection and the Ministry of Land, the national total percentages of soil heavy metal pollution exceeded 16.1%, in which slight, mild, moderate, and severe contamination of soils was, respectively, 11.2%, 2.3%, 1.5%, and 1.1%. Soil Cd and Pb pollution were ranked at the 1st and 5th, with pollution percentages being 7.0% and 1.5%, respectively (The Ministry of Environmental Protection and the Ministry of Land, 2014). Accumulation of heavy metals in soils has elevated heavy metal concentrations in crops, thus affecting the quantity and quality of crop production. A survey carried out by the Rice Product Quality Inspection

and Supervision Center, China's Ministry of Agriculture in 2002 showed that of the examined heavy metals in brown rice, Pb had the highest percentage (28.4%) exceeding the standard level, followed by Cd (10.3%) (Ying et al., 2010). Pan and his coworkers have also found about 10.0% of all rice grown and sold in China failed to meet the government standards for Cd (Zhen, 2008). It was estimated that China produced 12 million tons of brown rice contaminated with heavy metals per year, causing direct economic losses of more than 20 billion CNY (Zhou et al., 2006). Therefore, remediation of heavy metal contaminated soils is emergent and imperative.

A two-tiered strategy has been used to remedy soil contaminated by heavy metals (Zhou and Song, 2004; Adriano et al., 2004; Peng et al., 2009; Sun et al., 2013b). One is *in situ* stabilization of metals on soil particles (e.g. immobilization by chemical amendments and phytostabilization), and another is *ex situ* extraction or separation of metals from contaminated soils (e.g. phytoextraction, washing, flotation and land filling). However, scavenging heavy metal contaminated soils via conventional engineering methods is time-consuming and expensive. The *in situ* stabilization of metal elements by natural or synthetic additives such as phosphate compounds (Cao et al., 2002; Sukandar et al., 2009), alkaline compounds (Ma et al., 2012), clay minerals (Liang et al., 2013; Sun et al., 2013a,b), and biosolids (Tandy et al., 2009) has been considered as a promising alternative to the currently available

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remediation methods because of its cost-effectiveness and easy implementation as well as its application to a wide range of polluted sites (Garau et al., 2007). Adsorption, ion exchange, complexation, and precipitation are the major mechanisms involved in the conversion of soluble and potentially soluble forms of heavy metals to geochemically stable solid phases (Adriano et al., 2004; Cao et al., 2008), thus reducing heavy metal pool for plant uptake in soils.

However, no official protocols for assessing the effectiveness of soil remediation treated with *in situ* immobilization methods are available. Tessier sequential extraction procedure has been commonly applied because it provides information on the fractionation of heavy metals in different parts of the solid, which serves as a good compromise to obtain insight into the processes and mechanisms of element recoverability, retention, and release (Hisham and Randa, 2009; Al-Hwaiti et al., 2014). Biological factors may better indicate the environmental balance through the biotic indexes, derived from the observation of bioindicator species. Plant tests (e.g. growth response, metal bioaccumulation and biochemical, and physiological characteristics) are useful for the determination of the need for and the effectiveness of amendments (Zhou and Song, 2004). A decrease of antioxidant enzyme activities and protein contents along with an increase of malondialdehyde (MDA) has shown promising applications for the evaluation of plant responses to heavy metal exposures (Wang and Zhou, 2006). Soil enzyme and microbial activities are usually recommended as standard biological indicators of soil health, reflecting the adverse effects of metallic trace elements on soil quality (de Mora et al., 2005; Hinojosa et al., 2008; Sun et al., 2013a). Hence, combining physico-chemical and biological methods would provide a more thorough evaluation of remediation for heavy metal contaminated soils with chemical amendments (Adriano et al., 2004; Sukandar et al., 2009).

Bentonite, a 2:1 type of aluminosillicate, is a kind of expandable clay composed primarily of montmorillonite, characterized by high permanent negative charges and large specific surface area. It has been documented that bentonite is an efficient adsorbent for some heavy metals, especially for Pb and Cd (Karapinar and Donat, 2009; Hamidpour et al., 2010; Li et al., 2010).

Therefore, the objective of this study was to evaluate the chemical stabilization of Cd and Pb in contaminated soils by bentonite. Heavy metal fractions in soils and heavy metal concentrations in *Oryza sativa* L. were determined. Ecotoxicological evaluation parameters such as plant biochemical processes, plant growth responses, soil enzyme activities, and soil microbial community were also examined.

2. Materials and methods

2.1. Soil characteristics and plant culture

Surface soil (0–20 cm) was collected from agricultural fields in Tianjin, China. The soil samples passed through a 20-mesh sieve were used for physical and chemical analysis, and those passed through a 100-mesh sieve for determination of Cd and Pb concentrations. The soil was meadow burozem with 21.8% clay, 20.8% sand, and 57.4% silt. Soil pH, CEC, OM, total N, available P, available K, total Cdm and total Pb were, respectively, 8.2, 14.7 cmol kg^{-1}, 3.3%, 1300 mg kg^{-1}, 43.8 mg kg^{-1}, 103 mg kg^{-1}, 0.15 mg kg^{-1}, and 89 mg kg^{-1}.

Soil (6.0 kg, passed through 4 mm mesh) was placed in each plastic pot. A moderate contamination level was set at 5 mg Cd kg^{-1} and 2000 mg Pb kg^{-1} because the environmental quality standard (Grade III) for Cd and Pb in soils of China (GB 15618-1995) is 1.0 and 1000 mg kg^{-1}, respectively. Bentonite was incorporated into the contaminated soils at 0%, 0.5%, 1%, 3%, and 5%. Each treatment was performed in triplicate, and the treated soils were incubated for 5 weeks prior to growing rice.

Seeds of O. sativa L. (Jinyuan 47) were sterilized in 2% (v/v) H$_2$O$_2$ for 15 min, washed with tap water, and then soaked in water for 1 day. The soaked seeds were sowed in unpolluted soils. After 4 weeks, three seedlings of *O. sativa* L. of similar sizes (approximately 8 cm to 10 cm in height with 4 to 6 leaves) were transplanted in each contaminated soil. Soil water was maintained by adding tap water daily (no Cd detected) to 100% of the soil field water-holding capacity. A Petri dish was placed under each pot to collect potential leachates. After 150 days of growth, the plants were harvested, washed with tap water and then rinsed 3–4 times with deionized water. The harvested plants were separated into straw and brown rice, and oven-dried (75 °C) to a constant weight. All samples were ground to < 0.25 mm sieve with stainless steel mill for analysis.

2.2. Determinations of malondialdehyde (MDA), superoxide dismutase (SOD), peroxidase (POD), and soluble protein

About 0.500 g of the third leaf of *Oryza.sativa* L. was ground in an ice-cooled mortar with 5 mL of ice-cooled 50 mM Na-phosphate buffer (pH 7.8, containing 0.1 m MEDTA and 1% (w/v) polyvinylpyrrolidone). The homogenate was centrifuged at 10,000 rpm for 20 min at 4 °C. The supernatant was used for further determination (Wang and Zhou, 2006). Superoxide dismutase (SOD) activity was assayed as described by Sun et al. (2009a,b). One enzyme unit was defined as 50% inhibition of the colorimetric reaction. The activity of peroxidase (POD) was determined using guaiacol as substrate according to the method described by Wu and von Tiedemann (2002). Malondialdehyde (MDA) content was measured as described by Sun et al. (2009a,b) and expressed as nmol g^{-1} FW. Soluble protein (SP) concentration in the supernatant was estimated by the method of Wang and Zhou (2006) and expressed as mg g^{-1} FW.

2.3. Cadmium and Pb concentrations and their fractions

The plant and soil samples were digested with a solution of HNO$_3$-HClO$_4$ and HCl-HNO$_3$-HF-HClO$_4$, respectively, prior to determinations of Cd and Pb in solution.

Soil Cd and Pb fractionation was performed using sequential extraction of Tessier (Tessier et al., 1979). These fractions were: water soluble plus exchangeable fraction (SE) (1 mol/L MgCl$_2$ extractable), bound to carbonate or weakly specifically adsorbed (WSA) (1 M NaAc extractable), bound to Fe-Mn oxide fraction (OX) (0.04 M NH$_2$•HCl extractable), bound to organic fraction (OM) (30% H$_2$O$_2$ and 3.2 M NH$_4$Ac extractable), and residual fraction (RES, difference of total metal content minus the above four fractions).

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 concentrations of Cd and Pb in solution were determined by a flame atomic absorption spectrophotometer with a 1.3 nm spectral band width (Solaar M6, Thermo Fisher Scientific, USA).The wavelength for Cd and Pb analysis was 228.8 and 283.3 nm, respectively.

2.4. Soil enzyme activities and soil microorganisms

Urease activity was assayed with urea as substrate, incubating in 0.2 M phosphate buffer at pH 7.1 and 37 °C for 24 h. The residual urea was determined by a colorimetric method. The enzymatic activity is expressed as mg NH$_4$-N g^{-1} h^{-1} (Sun et al., 2013a,b). Catalase activity was analyzed by titration with KMnO$_4$ (Stępniewska et al., 2009), and expressed as mg KMnO$_4$ per gram of dry soil. Invertase activity was measured by incubating soil and sucrose at 37 °C for 24 h and then measuring glucose production with a colorimetric method, expressed as mg g^{-1} h^{-1} (Kandeler et al., 1999).

Soil microbial population (bacteria, fungi, and actinomycetes) was estimated by the dilution plate technique (three replicates for each dilution and soil dish) (Shen et al., 2005).