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Bioremediation of cadmium- and zinc-contaminated soil using *Rhodobacter sphaeroides*

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HIGHLIGHTS

- *Rhodobacter sphaeroides* had a remediation effect on soil contaminated with metals.
- Available metal fractions were transformed to less accessible and inert fractions.
- Cd phytoavailability was significantly reduced in treated soils.
- *Rhodobacter sphaeroides* bioremediation enhanced Zn/Cd ratio in wheat.

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ABSTRACT

Bioremediation using microorganisms is a promising technique to remediate soil contaminated with heavy metals. In this study, *Rhodobacter sphaeroides* was used to bioremediate soils contaminated with cadmium (Cd) and zinc (Zn). The study found that the treatment reduced the overall bioavailable fractions (e.g., exchangeable and carbonate bound phases) of Cd and Zn. More stable fractions (e.g., Fe-Mn oxide, organic bound, and residual phases (only for Zn)) increased after bioremediation. A wheat seedling experiment revealed that the phytoavailability of Cd was reduced after bioremediation using *R. sphaeroides*. After bioremediation, the exchangeable phases of Cd and Zn in soil were reduced by as much as 30.7% and 100.0%, respectively; the Cd levels in wheat leaf and root were reduced by as much as 62.3% and 47.2%, respectively. However, when the soils were contaminated with very high levels of Cd and Zn (Cd 54.97–65.33 mg kg^{-1}; Zn 813.4–964.8 mg kg^{-1}), bioremediation effects were not clear. The study also found that *R. sphaeroides* bioremediation in soil can enhance the Zn/Cd ratio in the harvested wheat leaf and root overall. This indicates potentially favorable application in agronomic practice and biofortification. Although remediation efficiency in highly contaminated soil was not significant, *R. sphaeroides* may be potentially and practically applied to the bioremediation of soils co-contaminated by Cd and Zn.

1. Introduction

Heavy metals contamination in soils is becoming an increasingly urgent problem throughout the world (Li and Zhou, 2012; Komarek et al., 2013; Bolan et al., 2014; Liang et al., 2014). Pedogenic processes and anthropogenic activities associated with industrial processes, manufacturing, domestic and industrial waste material disposal, and phosphate fertilizer application are the major sources of metal enrichment in soils (Wu et al., 2010; Boussen et al., 2013; Saifullah et al., 2013; Bolan et al., 2014; Sarwar et al., 2015). Cadmium (Cd) is a non-essential heavy metal that is toxic, cancerogenic, and teratogenic. It does not have a biological role in living tissue (Meda et al., 2007; Li and Zhou, 2012; Saifullah et al., 2013; Sarwar et al., 2015, 2017; Rebekić and Lončarić, 2016). Cd in soil is of great concern, because it threatens biodiversity, agricultural productivity, food safety, and human health as it is transmitted through the food chain (Saifullah et al., 2013; Sarwar et al., 2015; Tavarez et al., 2015). Zn is also a common heavy metal in contaminated soil. Unlike Cd, Zn is an important essential element, and has important regulatory roles in a number of biological processes, such as facilitating electron transfers for proteins and acting as co-factors of numerous enzymes (Sarwar et al., 2017). However, all heavy metals may cause negative ecological effects when they exceed toxic limits. For example, overdoses of Zn can cause harmful

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effects such as dizziness and fatigue (Sarwar et al., 2017).

Several studies have demonstrated that Zn can competitively inhibit Cd movement and accumulation in crops. As such, Zn fertilization has been regarded as a viable agronomic practice or biofortification approach to reduce Cd levels (Hart et al., 2002, 2005; Meda et al., 2007; Khoshgoftarmanesh et al., 2013; Saifullah et al., 2013; Fahad et al., 2015a; Sarwar et al., 2015; Tavarez et al., 2015). Thus, Zn fertilization is very likely to produce the co-contamination of Cd and Zn in soil, particularly when Zn fertilization is used to reduce Cd levels and increase Zn levels in Zn-deficient soil (Khoshgoftarmanesh et al., 2013; Fahad et al., 2015a, 2015b). It is common to find soil that is contaminated with both Cd and Zn. For example, one study found that Cd and Zn concentrations were as high as 42 and 4813 $mg\ kg^{-1}$, respectively, in soil in the Jalta mining area (Boussen et al., 2013). Thus, studying the co-contamination of Cd and Zn in soil has realistic and significant applications.

Remediating metal-contaminated soils is important because these soils generally cover large areas that are rendered ineligible for sustainable agriculture (Komarek et al., 2013; Ahemad, 2014; Chirakkara et al., 2016). Different physical, chemical, and biological techniques are used to remove heavy metals and metalloids from soils (Wu et al., 2010; Komarek et al., 2013; Bolan et al., 2014; Sarwar et al., 2017). In general, remediation strategies can be classified into two categories: mobilization and immobilization (stabilization), which can be used both in situ and ex situ (Vangronsveld et al., 2009; Bolan et al., 2014). Stabilization techniques have shown to be cost-efficient, safe, and the least destructive alternatives. These techniques include chemical stabilization (using different stabilizing amendments to reduce contaminant mobility, bioavailability and bioaccessibility), phytostabilization (using higher plants and associated microorganisms to immobilize contaminants in the root zone) and their combination (so-called aided phytostabilization) (Wu et al., 2010; Komarek et al., 2013; Bolan et al., 2014). Using plants and microbes is preferred with stabilization techniques, because of their cost-effectiveness, environmental friendliness, and fewer side effects (Vangronsveld et al., 2009; Wu et al., 2010; Dixit et al., 2015). Moreover, many studies have found that, microbes, especially rhizospheric microorganisms (e.g., mycorrhizal fungi, symbiotic bacteria and free living rhizobacteria), play an important roles in phytoremediation, and microbial-assisted phytoremediation has become a hot spot (Ahemad, 2014; Dixit et al., 2015; Sarwar et al., 2017). Microremediation itself is also an important strategy of soil bioremediation (Wu et al., 2010; Mani and Kumar, 2013). Furthermore, microorganism immobilization has significant advantages with respect to remediation cost and environmental compatibility. For example, it was reported that biostimulation costs were only about half of the costs of conventional stabilization (Mani and Kumar, 2013). Therefore, more scholars have focused on immobilizing heavy metals in soils using microorganisms.

Rhodobacter sphaeroides is a gram-negative, phototropic purple non-sulphur bacterium with several metabolic pathways depending on the growth conditions (Calvano et al., 2014). This bacterium has drawn great attention because of its ability to grow in both microaerobic and anaerobic light conditions. It uses different substrates as carbon and energy sources, and uses ammonium and/or nitrate as a nitrogen source. It may also use sulphide or thiosulphate as an electron donor under photosynthetic conditions (Madukasi et al., 2010; Calvano et al., 2014). *R. sphaeroides* has a high tolerance of abiotic stress conditions, including the presence of both organic and inorganic pollution (Li et al., 2016, 2017). Most importantly, this bacterium can decrease the mobility of heavy metals by generating sulfides (Bai et al., 2008; Jiang and Fan, 2008; Fan et al., 2012; Li et al., 2016, 2017). Sulfides have a very low solubility product constant and aid in heavy metal precipitation, recycling, and reuse (Muyzer and Stams, 2008; Kumar et al., 2014; Fonti et al., 2015). Thus, *R. sphaeroides* is widely used for environmental remediation, especially in wastewater treatment (Li et al., 2017). *R. sphaeroides* had been used to degrade different contaminants (e.g., phosphorus, atrazine, salts, radionuclide and heavy metal) sediment mud; studies have also evaluated its removal efficiency, impacting factors, mechanisms, and the phytotoxicity after bioremediation (Takeno et al., 1999; Du et al., 2011; Panwichian et al., 2012; Sasaki et al., 2012a, 2012b). Moreover, previous research has applied *R. sphaeroides* to restore soils contaminated with Cd and Pb (Fan et al., 2012; Li et al., 2016). However, there have been few studies concentrating on bioremediation of co-contamination of Cd and Zn or other heavy metals in soil using *R. sphaeroides*.

To address this knowledge gap, this study had the following objectives: 1) compare the bioremediation effects of *R. sphaeroides* on soils contaminated with different levels of Cd and Zn; 2) analyze the remediation efficiency and mechanisms associated with the chemical fractions of heavy metals and wheat phytoavailability; and 3) discuss the possible interactions between Zn and Cd.

2. Materials and methods

2.1. Strain

The bacterial strain used for soil bioremediation was isolated from the oil field injection water in DaQing, Heilongjiang Province, China. The strain was identified as *R. sphaeroides* based on its morphological and physical bio-chemical characteristics and its gene sequence of 16S rRNA (Fan et al., 2012; Li et al., 2016). Postgate C culture medium was used for strain enrichment and cultivation (Postgate, 1979).

2.2. Background soil

The background soil used for this study was collected from the Lianxiangqiao experimental field at the Chinese Academy of Agricultural Sciences in Beijing, China. After the soil was naturally air-dried, gravels and large organic scraps were removed from the sample. The sample was then sieved through a 0.83 mm nylon mesh. In general, the main heavy metals in this background soil were lower than background values found in Beijing soil (Table S1).

2.3. Preparation of spiked soils with different pollution level

After sieving pretreatment, each 1.5 kg background soil sample was sub-divided and placed in a 2.0 L glass beaker. In each beaker, different amounts of $Cd(NO_3)_2$ ($10.0\ mg\ mL^{-1}$) and $Zn(NO_3)_2$ ($100.0\ mg\ mL^{-1}$) spiked solutions were added to simulate different pollution levels in the soils. There were a total of eight test groups, named S1 (no Zn and Cd added), S2, S3, S4, S5, S6, S7 and S8, respectively. The beakers were sealed with preservative film, stirred once daily, and incubated at room temperature for 20 days, to reach adsorption equilibrium. Each test group had three duplicates. Table 1 lists the Cd and Zn concentrations in each test group.

2.4. Bioremediation experiment

The remediation tests were performed in the 2.0 L glass beakers. After 20 d spiking, 300 mL of the strain liquid (approximately 2×10^8 of *R. sphaeroides*, $CFU = 6.7 \times 10^5\ mL^{-1}$) (Fan et al., 2012) was directly added into each test group. Deionized water was then added to maintain 1800 mL of overlying water. After the soil was fully mixed with the strain, the beakers were sealed with