Remediation effect of cadmium contaminated soil by the combination of *Lactobacillus plantarum* and red mud

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**Abstract**: Bioaugmentation has been receiving much attention recently as an in-situ technology for the remediation of heavy metal polluted soils. To study the effects of the application of different dosages of *Lactobacillus plantarum*, red mud, and their mixture on cadmium-contaminated soil, the pot experiment was conducted to study the content of cadmium in garlic bolt, plant biomass, soil available Cd concentration, and soil physico-chemical property response. The results showed that: compared with the control, the application of *Lactobacillus plantarum* resulted in increase of available nitrogen, phosphorus and potassium in soils, while the addition of red mud only increased the content of soil available potassium. However, the biomass of garlic bolt was promoted by the application of *Lactobacillus plantarum*, red mud and their mixtures to different extend. *Lactobacillus plantarum* and red mud were applied alone or in combination could effectively reduce the contents of available Cd in the soil and Cd contents in plants, which was 32.4%~59.4% and 17.0%~44.3% lower as compared to the control, respectively, the combination of *Lactobacillus plantarum* and red mud with high dose could obtain best effect on the solidification of cadmium.

**1. Introduction**

As one of the major heavy metal pollution elements in the agricultural environment, cadmium (Cd) is mainly deposited into farmland soil through a large number of mining, the out-of-order letting of industrial waste gas, waste water and waste residue, the spraying of agricultural fertilizer and the dumping of daily garbage. It is difficult to be degraded and eventually enters the human body along with the gradual accumulation of soil-plants-animals, posing a threat to human health and survival [1]. Cadmium pollution in China not only has a wide range and deep degree, but also has serious harm. Its pollution control has become an urgent task of environmental governance, and the related treatment technology has become one of the focus issues in the field of environmental science. Due to the large area of cadmium contaminated soil in China, it is difficult and expensive to use traditional remediation methods. At present, there are three main methods for remediation of cadmium-contaminated soil in China and abroad: physical remediation, chemical remediation and biological remediation [2].

In recent years, heavy metal contaminated soil passivation and detoxification remediation technology has attracted much attention, which is operable and can be repaired while producing. The remediation effect of heavy metal passivation and detoxification can be achieved by applying some passivators with ion adsorption characteristics in heavy metal contaminated soil [3]. Previous studies showed that red
mud has stable chemical composition, very fine dispersion, high specific surface area, good adsorption performance and good stability in water medium. It is easy to adsorb metal ions in soil and water environment, and is an effective heavy metal passivation adsorbent [4]. However, excessive application of passivating remediation agents may cause secondary pollution, hinder the absorption of trace elements by plants, and affect the structure and physicochemical properties of soil, which will directly affect the continuous application of passivating remediation agents.

In view of the possible problems of chemical passivation repair agents, bioremediation has become a research hotspot. Using passivation bacteria to prevent plants from absorbing and accumulating heavy metals has become a research hotspot [5]. The bacterial surface has many functional groups that can combine with heavy metals to compete for heavy metals in soil. Lactic acid bacteria are the dominant bacteria in human intestinal tract, which are widely used in food processing and production, health products development, disease treatment and microecological agents. In recent years, more and more scholars have found that some lactic acid bacteria have more outstanding tolerance and adsorption performance to heavy metals [6].

Some scholars began to explore the effectiveness of combined use of bacteria and passivators. He et al. (2019) found that bacteria combined with sepiolite could significantly reduce the lead content in pepper and eggplant fruits and improve the quality of vegetables [7]. Chen et al. (2016) combined Neorhizobium huautlense T1-17 with vermiculite or peat, and found that compared with single treatment, the combined treatment could significantly reduce the content of lead and cadmium and the accumulation of heavy metals in pepper fruits [8].

In this study, a pot experiment was conducted to study the effects of different amendments on garlic bolt growth, heavy metal content and soil physico-chemical properties in Cd-contaminated soil by using red mud and Lactobacillus plantarum isolated from homemade enzymes as passivators. The study will provide theoretical basis and practical experience for the remediation of contaminated farmland and the safe production of vegetables in the future.

2. Materials and Methods

2.1. Experimental materials
The soil used in the pot experiment was artificially contaminated with Cd. The natural soil came from 0-20 cm topsoil in the suburb of Guiyang. About 2.5 kg portions of the soil were transferred to plastic pots (19 cm in diameter and 23 cm in height). Cd was added at a concentration of 5 mg·kg⁻¹ dry soil as an aqueous solution of CdCl₂·2.5H₂O. The garlic bolt seeds used for the experiment were purchased from the vegetable market in Guiyang. The red mud was taken from Guiyang Aluminium Plant. Lactobacillus plantarum was purified and isolated from the self-made enzyme samples in College of Food and Pharmaceutical Engineering, Guiyang University, the number of effective viable bacteria was higher than 2×10⁸ cfu/ml.

2.2. Experimental methods
Two materials as above mentioned were applied alone and in combination with various mixed modes at two application rates (the high dose was two folds of the low one). Application rates of a single material in low dose were 0.5% (w/w) for red mud, 1% (v/w) for Lactobacillus plantarum, respectively. A total of 7 treatments were involved in the pot experiment, and details of the treatments design were summarized in Table 1. The experimental design was in a completely randomized complete block with four treatments in quadruplicate. Full plant seeds were selected and planted directly in pots. The routine water management was performed. When the plants germinated to a height of about 5 cm, 10 plants were kept in each pot.
### Table 1 Experimental design of various treatments

| Treatment      | Red mud dose | Lactobacillus plantarum dose |
|----------------|--------------|------------------------------|
| CK             | 0            | 0                            |
| R1             | 0.5%         | 0                            |
| R2             | 0.1%         | 0                            |
| L1             | 0            | 1%                           |
| L2             | 0            | 2%                           |
| R1+L1          | 0.5%         | 1%                           |
| R2+L2          | 0.1%         | 2%                           |

During the harvesting stage, soil samples and aboveground samples of plants were collected for later analysis, and the plant weight were measured. The plant samples were digested with a 4:1 ratio of concentrated HNO₃ to HClO₄. The soil samples were digested using a concentrated mixture of HNO₃, HClO₄, and HF with a ratio of 6:2:2 for the analysis of total heavy metals. The available Cd was extracted from soil with 0.1mol·L⁻¹ hydrochloric acid. The Cd concentrations in solutions were determined by inductively coupled plasma optical emission spectrometry (ICP-OES). The physico-chemical property of soil, including pH, available nitrogen (AN), available phosphorus (AP), and available potassium (AK), was analyzed following protocol of Shi et al. [9].

2.3. Data processing

The experimental data were processed and plotted using Excel 2013, and the single-factor analysis of variance of the relevant data was performed by SPSS 22.0.

3. Results and analysis

3.1. Effects of various amendments on plant biomass

Fig. 1 showed that the application of various amendments alone or in combination had different effects on the biomass of the tested plants.

![Fig. 1 Effect of the various amendments on plant biomass](image)

It can be seen from the figure that, compared with the control, the addition of red mud and low dose treatment of Lactobacillus plantarum increased plant biomass slightly, but not significantly. While the plant biomass was significantly (P<0.05) affected by the addition of red mud-Lactobacillus plantarum mixtures, and displayed a positive dose-effect response, being the highest in the R2+L2 treatment, which was 33.2% higher as compared to the control.

3.2. Effects of various amendments on the soil physico-chemical property

Pictorial representation of physicochemical parameters of each soil treatment were presented in Fig.2 Overall, compared with the values for the control, the application of red mud could increase the pH, and AK contents of the soils, and the increase increased with the red mud dosage, however, a decline of AN and AP contents were obtained by the addition of red mud. Lactobacillus plantarum addition, either alone or in combination with red mud, lead to the elevation of AN, AP and AK contents. Among the
treatments, the maximum AN, AP and AK contents were obtained in the high dose treatment of *Lactobacillus plantarum* (L2), which were 1.50, 1.31 and 1.55 times of those under CK treatment, respectively.

3.3. Effects of various amendments on the available Cd content in soil and Cd contents in plants

The environmental risk of a heavy metal is more related to its bioavailability or available concentration (Ivanova, 2004). The available concentrations of Cd in soils were presented in Fig. 3. Obviously, as seen in Fig. 3, the available Cd contents in soils under all treatments were significantly lower compared to the control, indicating that all the treatment amendments could stabilize Cd and decrease the mobility of Cd in soils matrix. In addition, R2 + L2 treatment showed the best results in decreasing the available Cd, which was 59.4% lower as compared to CK, followed by R1+L1, R2, and R1 treatments.

With respect to the Cd contents in plants, all the amendment materials resulted in significant decline of Cd contents in plants, reduced by 17.0%-44.3% compared to that under the control treatment. Similar to the available Cd in the soil, the amendment materials for lowering the content of Cd in plants followed the order: R+L > R > L. However, the decrease of Cd contents in plants was generally less than that of available Cd in soil.
4. Discussion

In the present study, the amendments materials have various effects on the physico-chemical properties of soil, but all the treatment amendments could promote the biomass of plants to different extend. Red mud contains high nutrient elements such as Ca, K and P, the application of red mud elevated the available K content, and reduced the content of available N and available P in the soil. The reduction of available N by red mud is mainly due to the adsorption of red mud. Iron oxide and alumina in red mud can fix nutrients such as phosphorus in the soil, and reduce the content of available P in the soil [11]. Potassium is one of the essential nutrients for crop growth. Increasing the use of potassium in heavy metal contaminated soil can enhance the stress resistance of crops and promote the growth of crops [12]. The addition of Lactobacillus plantarum resulted in increase of available N, P, K in soil. On the one hand, lactic acid bacteria could increase soil enzyme activities such as soil urease and phosphatase, and then increase the content of soil available nutrients through enzymatic reflection [13]. On the other hand, Lactobacillus plantarum produce a variety of organic acids during the fermentation process. These organic acids mainly include lactic acid, acetic acid, citric acid, and phenyl lactic acid, etc. These acids can reduce the pH value while interacting with Fe, Al, Ca, and Mn plasma to dissolve insoluble phosphate [14]. It is also believed that the mechanism of microbial dissolution of phosphorus is the exchange of phosphate and protons. Lactobacillus plantarum produce a large number of protons during metabolic activities, which increase the acidity of the medium and dissolve phosphate [15].

The addition of red mud leaded to the elevation of soil pH. Red mud is generated after alkali leaching alumina bauxite residue, therefore alkalinity of red mud is stronger, the pH value of the selected red mud can be as high as 11.28, so the red mud addition to the soil improved the soil pH condition to a certain extent [16]. The improvement of soil pH value, makes the available forms of heavy metals into hydroxide precipitation, and carbonate combined, reduce the soil heavy metal mobility and biological toxicity effect [17]. Red mud is also abundant in iron-aluminum oxides with active adsorption sites on the surface for water soluble state and exchange state of metal ions, resulting in sequestration of available free metal ions to reduce the available fraction [18]. In this study, Lactobacillus plantarum could effectively reduce the content of available Cd in the soil and inhibit the absorption of Cd by plants. It is generally believed that the main mechanism is due to the adsorption of Cd by Lactobacillus plantarum. The combination of Lactic acid bacteria with metal ions is a complex process. Cd adsorption mechanisms of Lactobacillus plantarum might include complexation, ion exchange, physical adsorption (electrostatic interaction), precipitation and diffusion. hydroxyl (O—H), carboxyl (C=O), phosphoryl (P=O), amide (N—H) and hydrocarbonyl (C—H) groups were involved in biosorption process [6].

5. Conclusions

(1) The application of various amendments promoted the plant biomass to different degree, R2+L2 treatment obtained the maximum increase, which was 33.2% higher as compared to the control.

(2) Compared with the control, the application of Lactobacillus plantarum resulted in increase of available nitrogen, phosphorus and potassium in soils, while red mud addition only increased the content of soil available potassium.

(3) Lactobacillus plantarum and red mud were applied alone or in combination with different dose could effectively reduce the Cd contents in plants, and contents of available Cd in the soil, the addition of red mud-Lactobacillus plantarum mixtures with high dose had the best remediation effect for cadmium.

Acknowledgments

This research was financially supported by the special funding of Guiyang science and technology bureau and Guiyang University 【GYU-KY-[2021]】 , Youth Science and Technology Talent Growth Project of Education Department of Guizhou Province (KY [2018] 294), and the National Natural Science Foundation of China (No. 41803018).
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