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Effect of coexisting metal ions on bio-precipitation of Cu^{2+} phosphate by *Rahnella* sp. LRP3 and its stability in soil

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Abstract: The phosphate precipitation of heavy metal induced by microorganisms plays an important role in immobilising heavy metal in soil. However, there is little knowledge about the effect of coexisting metal ions on the induction of Cu phosphate mineral and its stability. In this paper, the Cu phosphate precipitations, coexisting with Pb^{2+} or Ca^{2+} induced by strain LRP3, were characterised, and the stabilisation of the induced phosphate precipitates was also studied. The coexistence of Cu with Pb or Ca decreased the removal efficiency of Cu^{2+} by 17.18% and 9.78%, respectively, indicating the competitive adsorption between cations. Strain LRP3 could induce a new phosphate mineral of $CuCa_{10}(PO_4)_7$ when coexisting with Ca and also generate the phosphate minerals of $Pb(H_2PO_4)_2$ and $Cu_3(PO_4)_2$ when coexisting with Pb. The Cu-Ca coprecipitate could enhance the stability of Cu in dilute acid solution and soil with or without a plant, whiles the Cu-Pb one showed the opposite effect. Also, the Cu-induced phosphate precipitates were relatively stable and not easy to be absorbed by Pakchoi (*Brassica rapa* var. *chinensis*). The results showed that the influence of coexisting metal ions should be considered when phosphate mineralisation technology is used to immobilise heavy metals in the environment.

Keywords: microbially induced phosphate coprecipitation; heavy metal immobilisation; Pakchoi; *Rahnella* sp. LRP3

Copper (Cu) is a common and natural trace element in all environmental areas, including soil, air, water, and sediment. It plays a key role in plant respiration, photosynthesis, and some superoxide dismutase processes (Eom et al. 2019). However, a high concentration of Cu in the soil leads to plant toxicity, which induces the dispersion of metal particles through wind erosion. One of the main routes for impregnating these heavy metals by humans is consuming plants grown on these contaminated soils. These plants can draw these substances through their roots and accumulate them in the various organs (roots, stems, leaves) (Mench et al. 2006). Therefore, it is urgent to immobilise Cu in the soil to reduce the available content of Cu in soil and the absorption and enrichment of Cu by the plant.

In recent years, several studies have been dedicated to using microbial-induced phosphate precipitation (MIPP) techniques to immobilise heavy metals in the environment. The MIPP biomineralisation process presents a great potential to remediate polluted soil efficiently due to its simplicity in operation, low cost, green and ecofriendly process compared to traditional technologies (Abatenh et al. 2017, Jiang et al. 2020). For example, the MIPP products could adsorb or incorporate heavy metal ions into their crystal structure (Sowmya et al. 2014, Chen et al. 2016, Tu et al. 2019). Besides, many heavy metals (Pb, Cu, U, Zn, Cd) could be immobilised through the MIPP process in soil (Naik et al. 2013, Liang et al. 2016, Qian and Zhan 2016, Nie et al. 2017, Zhao et al. 2019).

Various studies have shown that some microorganisms can immobilise Cu through phosphate mineralisation, but it is often mainly for single Cu (Olayinka and Babalola 2001, Jerden Jr. et al. 2003, Mignardi et al. 2012). It has been reported that microorganisms

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can coprecipitate heavy metals and calcium through isomorphous replacement inducing carbonate or phosphate precipitation (Klas et al. 2011, Baldermann et al. 2019). The coexistence of calcium ions with heavy metals can affect minerals' crystal formation and morphology (Chen et al. 2016), the morphology of minerals, and the stability of phosphate mineralisation products, especially under the coexistence of Cu^{2+} and Ca^{2+}.

Moreover, most of the studies have been focused on the induction mechanism and biomineral characterisation. Therefore, little attention has been paid to the stability of induced precipitation in the environment. The previous studies have shown that soil acidification and plant roots significantly impact heavy metal availability in soil (Rieuwerts et al. 1998, Ginocchio et al. 2002, Wang et al. 2006, Farrag et al. 2012, Nadgórska-Socha et al. 2013, Adamczyk-Szabela et al. 2015). Thus, understanding the stability and activity characteristics of induced mineralisation products in the soil is also necessary to promote the practical application of MIPP technology in Cu-contaminated environments.

The bacterial strain *Rahnella* sp. LRP3 has been shown as an indigenous beneficial bacterium that can immobilise Cu through the phosphate mineralisation process (Zhao et al. 2019). To better use strain LRP3 for Cu with other metals contaminated soil remediation, we further studied the effect of Pb or Ca coexistence with Cu on the crystalline form and morphology of phosphate coprecipitate. We also carried out an acid dissolution experiment and pot experiment to study the effect of metal ions' coexistence on the stability of Cu in the induced coprecipitate. The results in the current study are expected to provide a theoretical basis and technical support for practical applications of MIPP technology based on *Rahnella* sp. LRP3.

MATERIAL AND METHODS

Bacterial strain and soil sample

The bacterial strain used in this study was identified as *Rahnella* sp. LRP3, which could induce phosphate precipitation of Cu (Zhao et al. 2019). The bacterial culture was made by inoculating bacterial colonies into nutrient broth phytic (NBP) medium (containing 3 g/L beef extract, 5 g/L NaCl, 5 g/L peptones, 2 g/L sodium phytic acid, and pH adjusted to 7.0–7.2) (Zhao et al. 2019).

The soil sample used in the current study was dark brown soil, which was collected at a depth of 0–20 cm from the plant production field of Jilin Agricultural University, Jilin province, China. The samples were dried and sieved through 2 mm before analysis. One part was used to determine physical and chemical properties and potted cabbage, and the other part was ground through a 0.15 mm sieve to determine total Cu in soil. The soil composition was as follows: 5.05% of total organic carbon (C), 180.2 mg/kg of total nitrogen (N), 67.9 mg/kg of total phosphorus (P), and 384.1 mg/kg of total potassium (K). The total and available Cu contents were 68.40 mg/kg and 11.19 mg/kg, respectively, with soil pH 6.65.

Cu removal efficiency analysis under the influence of coexisting heavy metals (Ca, Pb)

The heavy metals stocks were prepared with 100 mL of 0.5 mol/L $CuCl_2$, 0.5 mol/L $CaCl_2$, and 0.5 mol/L $Pb(NO_3)_2$ solutions, respectively, to use for the following experiments. 200 mL of NBP medium (containing 2% of bacterial suspension) was incubated at 160 rpm and 25 °C for 24 h as a bacterial culture. 5 mL of coexisting Cu-Ca and Cu-Pb with the ratio of 1:1 were added to the bacterial culture, respectively, and stored at a motionless condition. The upper liquid was collected and filtered through the 0.45 μm membrane to determine the Cu^{2+} concentrations at regular intervals (0, 15, 30, and 60 h).

$$\text{Removal efficiency (\%)} = (C_0 - C_t)/C_0 \times 100$$

where: C_0 and C_t – concentration at initial and at time t of heavy metals ions (mg/L).

Cu-phosphate precipitation formation under the influence of coexisting heavy metals (Ca, Pb)

The precipitates formed at the bottom of solutions in section 2.2 were used in this experiment. After 2 h at motionless condition, these precipitates were collected and dried at 50 °C in an oven for 48 h. All the precipitate samples have analysed the characteristics using scanning electron microscope-energy dispersive spectroscopy (SEM-EDS) and X-ray diffraction (XRD).

The stability of Cu-phosphate precipitates assays

The dissolution of phosphate precipitates in diluted acid. The weak acid in this experiment was prepared using 0.5 mol/L of acetic acid to adjust 100 mL of acid solution with pH 4.0. An aliquot of 1 g phosphate precipitates was added to 100 mL of

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the diluted acid solution, respectively, and shaken at 25 °C, 160 rpm. After 0.5 h and 4 h, the upper liquid was collected and filtered through a 0.45 µm membrane to determine Cu^{2+} concentration. The dissolution rate was calculated as follow:

$$\text{Dissolution ratio} = (C_0 \times V)/(C_t \times m) \times 100$$

Where: C_0 – dissolution of in dilute acid solutions (mg/L); V – volume of dilute acid solution (L); C_t – heavy metal content in precipitates (mg/kg); m – weight of added precipitates (kg).

Soil cultivation test. The three Cu-containing phosphate precipitates were added to the soil samples (the total amount of Cu in the soil ~ 280.0 ± 3.0 mg/kg). After mixing them, 20.0 g of soil was incubated in a 35 mm diameter plate in an incubator with constant temperature (28 °C) and humidity. The samples were collected at the beginning, on the 10th and 30th days of the cultivation, and the available Cu content was determined.

Pot experiments. The plastic pots 20 cm tall with a 20 cm mouth opening diameter were used in the experiment. The pots had drainage and were filled with 1.0 kg of soil samples. Three pots of soil were supplemented uniformly with different amounts of phosphate precipitates to make the total Cu content in the soil reach 280.0 ± 3.0 mg/kg. The pot of original soil without phosphate precipitation is used as a control. All the pots were incubated in a plastic greenhouse in the plant production base of Jilin Agricultural University, and the indoor temperature was 25 ± 2 °C. The period harvest time was 40 days of incubation, and the experiment was repeated three times.

Before planting, the Pakchoi seeds were soaked in 0.9% saline to filter out plump and uniform seeds and then sowed. Ten seeds of Pakchoi were then planted in each pot separately. The moisture content of 60% water holding capacity was adjusted during the growth of Pakchoi. The seedlings were thinned twice, and five seedlings finally grew in each pot. The rhizosphere soil of all the plants is collected during harvest. After cleaning, the leaves and roots of Pakchoi wiped the surface moisture with paper, then dried them in an oven at 105 °C for 30 min and continued drying them to constant weight at 75 °C. All the samples were stored in a ziplock bag in a desiccator to determine Cu content; the soil samples were collected simultaneously, then air-dried and sieved to determine Cu content.

Analytical method

The determination of Cu content. Determination of total Cu content in soil and plant tissues: 0.1 g of the sieved soil sample was put into the tube with 6 mL hydrochloric acid, 2 mL pure nitric acid, and 1 mL pure hydrofluoric acid, then digested in microwave digestion (MARS6, CEM, Germany); 0.3 g of the sieved plant tissue sample supplemented with 10 mL of pure nitric acid, was also digested in microwave digestion. After that, the digested samples were heated at 180 °C to reduce their volume in the tube by about 1 mL, then washed the tube wall with 20 mL of nitric acid solution (2%). After cooling, these samples were transferred to a volumetric flask and diluted to 25 mL of volume. The collected supernatants were used to analyse Cu content using atomic absorption spectrometry (AAS). During the determination, national standard soil samples [GB W07453 (GSS-24)] and national standard vegetable samples [GB W10015 (GSB-5)] were used as analysis quality control.

Determination of available Cu content: The extraction method using diethylenetriamine penta-acetic acid (DTPA) as the extractant was conducted as described by Zhao et al. (2019). The supernatant collected after extraction was passed through a 0.45 µm filter membrane and analysed using an atomic absorption spectrometer (Jiangsu Skyray Instrument Co., Ltd, Jiangsu, China).

The characteristics of phosphate precipitates. All the phosphate precipitate samples were dried at 50 °C. A part was ground through a 300-mesh sieve in an agate mortar for X-ray diffraction analysis to identify the characteristic of these precipitates described by Zhao et al. (2019). The heavy metals precipitates were compared with the mineral crystal database from the International Centre for Diffraction Data (ICDD) to identify their formula. The remaining part of the samples was examined the morphology and composition of the samples using the SEM-EDS system (X-550, Shimadzu, Kyoto, Japan), according to Zhao et al. (2019), with minor modification.

Statistical analysis

All experiments were completed with three replicates. The Excel 2017 software (Washington, USA) was used to draw the graphs, and the XRD pattern was analysed using Jade5 software (California, USA). Data were expressed as mean ± standard deviation (n = 3), and the ANOVA was used to analyse

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the significant difference of the treatment factors. Statistical analysis was performed using SPSS 25 software (Chicago, USA).

RESULTS AND DISCUSSION

The effect of Pb and Ca on the removal of Cu in the bacterial culture solution

The effect of coexisting metal ions on the removal of Cu^{2+} in solution (Figure 1) revealed that Cu^{2+} could be removed rapidly from Cu bacterial culture alone or with Ca and Pb within 15 min. The removal efficiencies of Cu in the bacterial culture alone and together with Ca and Pb were 66.3, 55.3, and 60.9%, respectively. After that, the removal efficiency of Cu^{2+} without the addition of Ca or Pb was significantly higher than the one with the coexistence of Ca or Pb. Besides, the Cu removal efficiencies in these three solutions were stable after 30 min.

The order of Cu removal with the coexisting of other heavy metals, is as follows: Cu > Cu-Ca > Cu-Pb. After the interaction between metal and the active groups on the cell wall, metal can be removed from the solution by forming complex groups on the cell surface. Carboxyl, amino, thiol, and hydroxyl are some of these groups. Phosphate and hydroxycarboxyl can interact with heavy metal ions coordinated (Sag and Kutsal 2001). The competition of metal cations to bind with some functional groups (phosphate, carboxyl, etc.) in the bacterial culture solution (Konhauser and Riding 2012) might be the reason for the decrease in the Cu^{2+} removal rate. A similar result was reported by Ren et al. (2018), who proved competitive reactions between heavy metals in the solution with phosphate. However, the removal rates of Cu in all three solutions were high (over 60%), which indicated that strain LRP3 could have the ability to immobilise Cu in the coexisting condition with other metals.

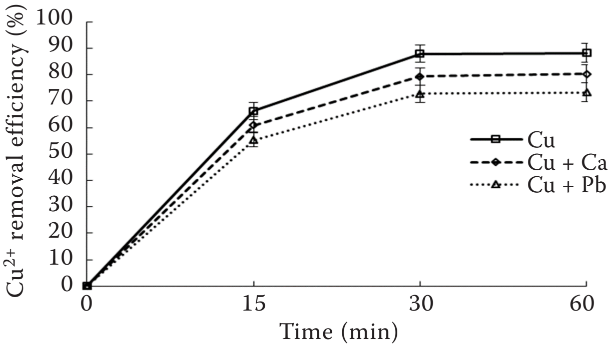

Figure 1. The effect of coexisting ions on the removal of Cu by bacterial culture of strain LRP3

Influence of Pb and Ca on the Cu phosphate precipitation in the bacterial culture solution

Initially, it was considered that metals might be deposited inside cells or on cell walls by sorption-precipitation at higher pH values (Javanbakht et al. 2014). Precipitation can be influenced by or unaffected by cellular metabolism (Ahalya et al. 2003). In the former situation, metal removal from solution is frequently linked to a microorganism active defence mechanism. Figure 2 shows the essential characteristics of Cu-phosphate precipitation induced by strain LRP3 in the bacterial culture solution with and without the coexisting heavy metals (Pb, Ca). It can be seen that the main diffraction peak position of a single Cu phosphate precipitation coincided with the diffraction peak crystal plane of the PDF card (37-0483), which corresponds with the molecular formula of $Cu_3(OH)_3PO_4$ (Figure 2A). In the solution with the presence of Cu-Pb coexisting, the main diffraction peaks of phosphate precipitation coincided with the crystal planes of two minerals $Pb(H_2PO_4)_2$ and $Cu_3(PO_4)_2$ when compared with the standard PDF cards (43-0524) and (36-0203), respectively (Figure 2B). However, in the coexisting solution of Cu-Ca, the main diffraction peak of the phosphate precipitate formed was consistent with the peak crystal plane in the standard PDF card (47-0899), which corresponds to the molecular formula of $CuCa_{10}(PO_4)_7$ (Figure 2C).

Besides, the SEM analysis of these three samples of phosphate precipitates showed that the one in the solution with the presence of a single Cu was a regular spherical shape and arranged in a more orderly arrangement (Figure 2D). But the precipitate of Cu-Pb was a non-regular spherical shape (Figure 2E), and the precipitate of Cu-Ca was a spherical shape combined from many small cubes (Figure 2F). The energy-dispersive spectroscopy (EDS) analysis results confirmed the formation of the phosphate precipitates in three bacterial culture solutions with and without the coexisting of Pb and Ca. Figure 2D showed the phosphate precipitate of single Cu was mainly composed of Cu, O, P, and H elements. The Cu-Pb precipitate mostly contained Cu, Pb, P, O, and H elements (Figure 2E), and the Cu-Ca precipitate mainly comprised Cu, Ca, P, and O elements (Figure 2F).

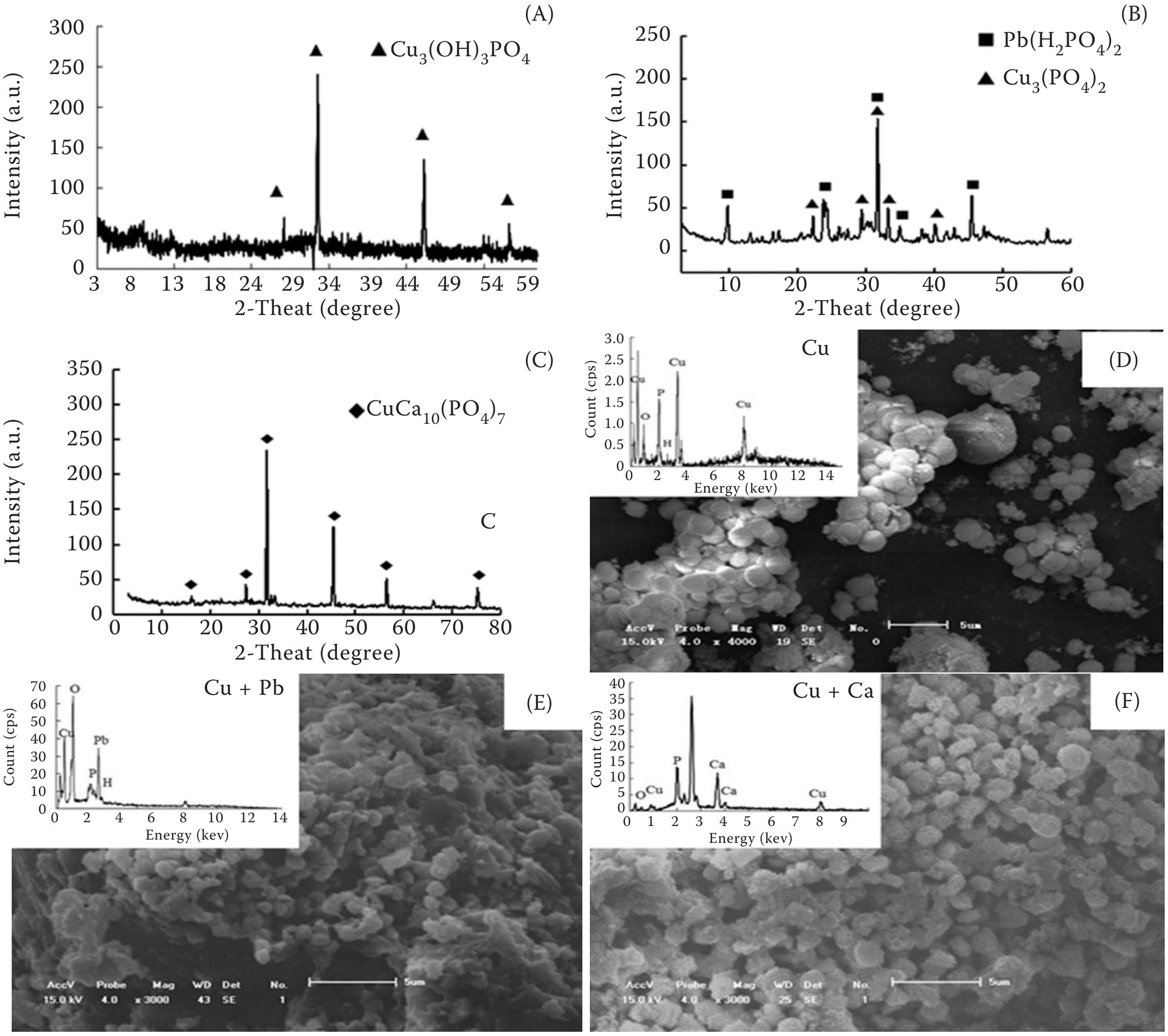

Figure 2. The characteristics of Cu-containing precipitates formed by strain LRP3 in three solutions. The XRD analysis of (A) Cu-containing precipitates in Cu solution; (B) Cu-containing precipitates in Cu-Pb solution; (C) Cu-containing precipitates in Cu-Ca solution; the SEM-EDS analysis of (D) Cu-containing precipitates in Cu solution; (E) Cu-containing precipitates in Cu-Pb solution, and (F) Cu-containing precipitates in Cu-Ca solution

These results showed that the crystalline form of Cu phosphate precipitate induced by strain LRP3 was affected, from $Cu_3(OH)_3PO_4$ in a single precipitate to $Cu_3(PO_4)_2$ and $Pb(H_2PO_4)_2$ when coexisting with Pb; and to a renewed phosphate precipitate ($CuCa_{10}(PO_4)_7$) containing both Cu and Ca was formed when coexisting with Ca. The interaction mechanism between strain LRP3 and metal ions is well discussed in our previous article (Zhao et al. 2019), which reported that the strain LRP3 could induce heavy metal phosphate precipitation formation through bacterial cells. First, the bacterial cells or extracellular secretions adsorb heavy metals, and the phosphate in the solution combines with heavy metals to form crystals. Chen et al. (2016) reported that the Pb(II) in the bacterial culture of *Bacillus cereus* 12-2 could be transformed into rod-shaped $Ca_5Pb_{15}(OH)_4(PO_4)_{12}$ in the presence of Ca in the solution. Simon and Biermann (2007) also demonstrated that the autunite $Ca(UO_2)_2(PO_4)_2$ crystal or chernikovite $H_2(UO_2)_2(PO_4)_2$ crystal was formed by reaction with hydroxylapatite. The change of environmental conditions such as coexisting ions would highly affect the heavy metal quantity and its toxicity in the soil, therefore directly impacting the biomineralisation efficiency of MIPP leading to changes in the composition, size, and shape of final crystals (Lin et al. 2016, Maity et al. 2019, Wei et al. 2019).

The dissolution of Cu^{2+} in phosphate precipitates by dilute acid

Further studies showed that the three types of phosphate mineral of Cu affect the dissolution efficiency of Cu in the dilute acid solution. As shown in Figure 3, the dissolution efficiency of Cu in the Cu-Pb phosphate mineral was significantly high at half an hour and 4 h times compared to the ones formed by single Cu precipitation and by coprecipitation of Cu-Ca. However, the dissolution rate of Cu in Cu-Pb and Cu precipitates remained relatively high after 4 h ($P < 0.05$). In comparison, there is no significance observed in the one formed by Cu-Ca precipitation (at 30 min and 4 h).

The results showed that the dissolution of Cu in the solution was different with different forms of Cu-phosphate precipitates. The order of Cu dissolution efficiencies is as follows: Cu + Ca < Cu < Cu + Pb. This result indicated that the Cu-Ca precipitate mineral was most stable in diluted acid solution than the Cu-Pb precipitate mineral, which was easy to dissolve in the diluted acid solution followed by a single Cu precipitate. This phenomenon could be explained by the presence of different phosphate minerals in the solution. The structure and morphology of mineral crystals could affect mineral stability, which leads to these various dissolved capacities (Zhao et al. 2020). However, in all three treatments, the Cu dissolution efficiencies were less than 2.5%, a small amount of Cu was dissolved. Nzihou and Sharrock (2010) demonstrated that the metal phosphate precipitate was very stable over geological time because of its low solubility products. These products were thermodynamically stable over a broad range of environmental conditions and precipitated rapidly, thus ensuring long-term immobilisation of metal pollutants. Besides, the presence of Ca^{2+} could promote the rapidly metal precipitation as a mineral phase, which is hardly dissolved (Nzihou and Sharrock 2010). This indicated that the Cu-phosphate precipitates induced by strain LRP3 could be used to remediate the coexisting heavy metals contaminated, even in acidic soil.

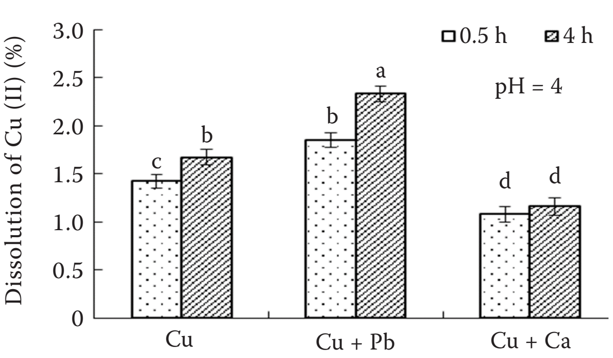

Figure 3. The dissolution of Cu-containing phosphate precipitation in dilute acetic acid solution. Error bars represent standard deviation ($n = 3$). The letters a, b, c, etc., represent the significantly different based on Duncan's test result ($P < 0.05$)

The stability of Cu phosphate precipitates in soil

Cu stability in the soil is strongly pH-dependent; mobility increases as pH decreases. Carbonates, phosphates, and clays can reduce Cu mobility in soil (Jalali and Moradi 2013). Figure 4 shows the content of available Cu in different treated soil (without and with coexisting Ca and Pb) at different times. The content of available Cu in the treated soil with Cu-Pb precipitate addition increased over time by 15.89% after 30 days ($P < 0.05$). However, in the soil treated by adding single Cu and Cu-Ca phosphate precipitates, although the available Cu content in the soil slightly decreased with the time increase, there was no significant change in the available Cu content after 30 min of incubation.

The above results indicate that Cu in Cu-Pb treatment was easy to activate and release, while Cu in single Cu treatment and Cu-Ca treatment was not easy to activate. This stability order is positively relevant to the result of the dissolution rate. Ren et al. (2018) indicated that the stability of heavy metals from single-metal-contaminated soil was higher than in coexisting-metal-contaminated soil, and Pb was a vital metal in the competitive stabilisation with other metals. Besides, in all three treatments, the proportions of available Cu in soil were small (less than 15%) compared with the results reported by Huang et al. (2020). Their research work reported that with the treatment of 200 mg/kg Cu in total, the exchangeable Cu was more than 25% in both rhizosphere and non-rhizosphere soils. This indicated that it could use the remediated soil, which contained coexisting heavy metals, in the presence of strain LRP3 for planting.

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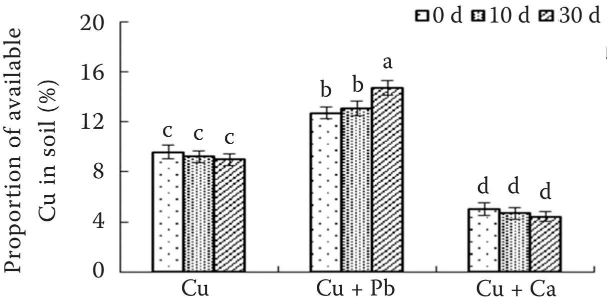

Figure 4. The proportion of the available Cu in the soil after 0, 10, and 30 days of incubation. Each value is the mean of triplicates. Error bars represent standard deviation. The letters a, b, c, etc., represent the significantly different based on Duncan's test result ($P < 0.05$)

Stability and transmission of Cu in phosphate precipitates in case of Pakchoi planting

Variation of Cu accumulation in Pakchoi. The untreated soil and the soil treated with three kinds of Cu-containing phosphate precipitates were used to carry out the pot experiment of Pakchoi. The use of phosphates is a standard method to reduce the mobility of heavy metals in contaminated soils. In particular, it is considered effective as an inexpensive and essential method for treating lead-contaminated soils (Ruby et al. 1994, Mignardi et al. 2012), but it is also beneficial for other heavy metals like copper. Thus the enrichment efficiencies of Cu by Pakchoi were calculated to assess the absorption ability of Cu by Pakchoi and the stability of Cu in soil. The result in Figure 5A shows that in both leaves and roots, the enrichment efficiencies of Cu in soil treated with three Cu-containing phosphate precipitates were reduced compared with the untreated soil. In comparison with the enrichment efficiency of Cu in leaves of Pakchoi grew in untreated soil, the ones that grew in the treated soil with Cu, Cu-Pb, and Cu-Ca phosphate precipitates decreased by 52.70, 36.49, and 60.81% ($P < 0.05$), respectively; and in the roots, these coefficients decreased by 54.07, 41.48 and 61.48% ($P < 0.05$), respectively. These results showed that the enrichment level of Cu in Pakchoi was in the following order: control sample > Cu-Pb > Cu > Cu-Ca, which indicated that the Cu-containing phosphate precipitates induced by the strain LRP3 was stable in the soil and not easy to be absorbed by plants. Also, the presence of Ca coexisting could promote the stability of Cu-mineral in soil, while the presence of Pb coexisting increased the mobility of Cu in the soil, which led to more absorption of Cu by Pakchoi.

Besides, the transmission capacity was used to determine the accumulation of Cu in Pakchoi. The results showed that these efficiencies were higher in three treatments (Cu, Cu-Pb, and Cu-Ca) than in the control sample (CK) according to the following order: CK < Cu-Ca < Cu < Cu-Pb (Figure 5B). This demonstrated that some of the absorbed Cu was easier transferred from root to leave of Pakchoi in three treated soil than in the control soil sample. It is perhaps because of the increase of Cu concentrations in the three treatments. Compared to the one in Cu and Cu-Ca treatments, the high transfer of Cu content from root to leaves in Cu-Pb treatment could be due to the available Cu presented in the soil or the effect of coexisting heavy metal on the metal retention in the root. This result was in contrast with the previous study reported by Nazarian et al. (2016), which demonstrated that transmission of

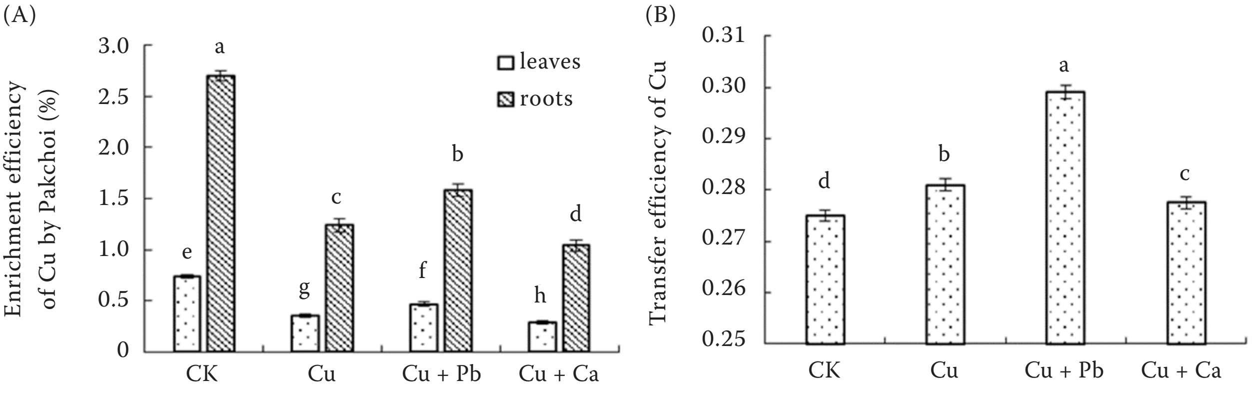

Figure 5. (A) The enrichment efficiencies of Cu in roots and leaves of Pakchoi; (B) the transfer efficiency of Cu concentration from root to leaves. Each value is the mean of triplicates. Error bars represent standard deviation. The letters a, b, c, etc., represent the significantly different based on Duncan's test result ($P < 0.05$)

cadmium from the root to the stem was decreased by increasing the cadmium quantity. Gao et al. (2013) reported that by increasing cadmium concentrations in vermilion, the cadmium concentration in roots and leaves was also increased.

However, the result showed that all the transfer efficiencies were small (less than 0.31), indicating that the Pakchoi roots could accumulate the majority of Cu and prevent heavy metal translocation to other growing parts of Pakchoi. The previous studies have proved similar results (Nouri et al. 2009, de Souza Costa et al. 2012, Huang et al. 2020). Martínez-Alcalá et al. (2010) also reported that heavy metal accumulation by *Lupinus albus* primarily reflected the solubility of the metals in the soil with lower values in the shoot than in the rice roots. Han et al. (2020) demonstrated that the soil treated with strains N3 and H12 reduced the Cd and Pb levels in the roots and leaves of Pakchoi, and the metals absorbed by the plant were higher in the roots than in the leaves. Besides, the soil treatment with Cu-Ca always showed outstanding efficiency compared to the control condition and other treatments. This might be because of the stability of the mineral precipitated by Cu and Ca or the promotion of coexisting ions (Ca^{2+}) to the immobilisation of Cu in soil. However, this metabolism is complicated and needs further study to confirm and clearly explain this phenomenon.

Variation of the available Cu in rhizosphere soil. The highly significant associations between pH and Cu in the rhizosphere suggest that root-induced alkalisation regulates Cu depletion. Metal depletion in the rhizosphere, on the other hand, is commonly thought to be caused by root uptake, which is thought to be far greater than the soil's ability to resupply metals to roots (Bravin et al. 2010). The ratio of available Cu to the total Cu in the rhizosphere soil of Pakchoi after potting is shown in Figure 6. The percentage of available Cu over the total Cu in the rhizosphere soils treated with three kinds of Cu phosphate precipitates is lower than that of the untreated soil. The addition of Cu, Cu-Pb, and Cu-Ca phosphate precipitates to the soil reduces the proportion of available Cu in rhizosphere soil by 45.83, 22.29, and 52.74%, respectively ($P < 0.05$). These data were significantly and positively correlated with the percentage of Cu uptake into Pakchoi.

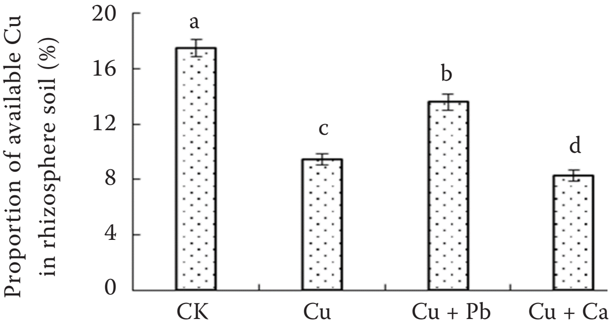

Figure 6. The proportion of the available Cu to the total Cu of the rhizosphere soil. Each value is the mean of triplicates. Error bars represent standard deviation. The letters a, b, c, etc., represent the significantly different based on Duncan's test result ($P < 0.05$)

The results indicated that the three kinds of phosphate precipitates induced by strain LRP3 could immobilise Cu and reduce the content of available Cu in the soil. Especially, in comparison with the phosphate precipitate of single Cu, the phosphate precipitate of Cu-Ca coexisting could promote the immobilisation of Cu in soil, which means reducing the available Cu in soil affects its absorption by the plant. In contrast, the phosphate precipitate of Cu-Pb decreased the stability of Cu and increased the available Cu in soil. The influence of phosphate on Cu availability could be due to phosphate being adsorbed by soil colloids. Sequioxides, for example, have an exceptionally high affinity for phosphate (Huang et al. 2003).

Our findings suggested that the interaction between coexisting ions and the activation of phosphate minerals by plant growth should be considered a means in the remediation of Cu polluted soil by microbial-induced phosphate mineralisation. Further studies may be necessary to determine the specific role of coexisting metal ions in the remediation of heavy metal contaminated agricultural soils.

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