



The Effect of Calcium Source on Pb and Cu Remediation Using Enzyme-Induced Carbonate Precipitation

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Specialty section:

This article was submitted to
Bioprocess Engineering,
a section of the journal
Frontiers in Bioengineering and
Biotechnology

Received: 06 January 2022

Accepted: 24 January 2022

Published: 11 February 2022

Citation:

Wang L, Cheng W-C and Xue Z-F
(2022) The Effect of Calcium Source on
Pb and Cu Remediation Using
Enzyme-Induced
Carbonate Precipitation.
Front. Bioeng. Biotechnol. 10:849631.
doi: 10.3389/fbioe.2022.849631

Heavy metal contamination not only causes threat to human health but also raises sustainable development concerns. The use of traditional methods to remediate heavy metal contamination is however time-consuming, and the remediation efficiency may not meet the requirements as expected. The present study conducted a series of test tube experiments to investigate the effect of calcium source on the lead and copper removals. In addition to the test tube experiments, numerical simulations were performed using Visual MINTEQ software package considering different degrees of urea hydrolysis derived from the experiments. The remediation efficiency degrades when NH_4^+ and OH^- concentrations are not sufficient to precipitate the majority of Pb^{2+} and Cu^{2+} . It also degrades when CaO turns pH into highly alkaline conditions. The numerical simulations do not take the dissolution of precipitation into account and therefore overestimate the remediation efficiency when subjected to lower $\text{Pb}(\text{NO}_3)_2$ or $\text{Cu}(\text{NO}_3)_2$ concentrations. The findings highlight the potential of applying the enzyme-induced carbonate precipitation to lead and copper remediations.

Keywords: heavy metal, biomineralization, urease enzyme, urea hydrolysis, remediation efficiency

INTRODUCTION

Heavy metal contamination not only raises sustainable concerns but also causes threats to human health and plants (Kumari et al., 2016; Jiang et al., 2019; Rahman et al., 2020; Chen et al., 2021a; Tan et al., 2022). In the past few decades, remediation methods including replacement, soil flushing, electrokinetic remediation, chemical precipitation, ion exchange, and phytoremediation were developed (Mena et al., 2016; Kumari et al., 2017; Kumari et al., 2018; Chen and Achal, 2019; Duarte-Nass et al., 2020; Ahenkorah et al., 2021; Hu L. et al., 2021; Jiang et al., 2021). However, they are usually time-consuming and may cause secondary pollution (Achal et al., 2012; Nancharaiah et al., 2016; Kappaun et al., 2018; Xue et al., 2022; Yan et al., 2021; Duan et al., 2021a; Cheng et al., 2021; Duan et al., 2021b). Furthermore, heavy metal ions may be re-released into surrounding environments when subjected to the change in environmental conditions (Hu et al., 2021b; Bai et al., 2017; Bai et al., 2021; Xue et al., 2021). In light of this, a sustainable countermeasure that is able to immobilize heavy metal ions using an environmental-friendly manner is considered of great necessity. Enzyme-induced carbonate precipitation (EICP) is a novel biogeotechnical technique and has been widely applied to strengthen calcareous sands (Ahenkorah et al., 2021; Wei et al., 2021; Yuan et al., 2021; Chen

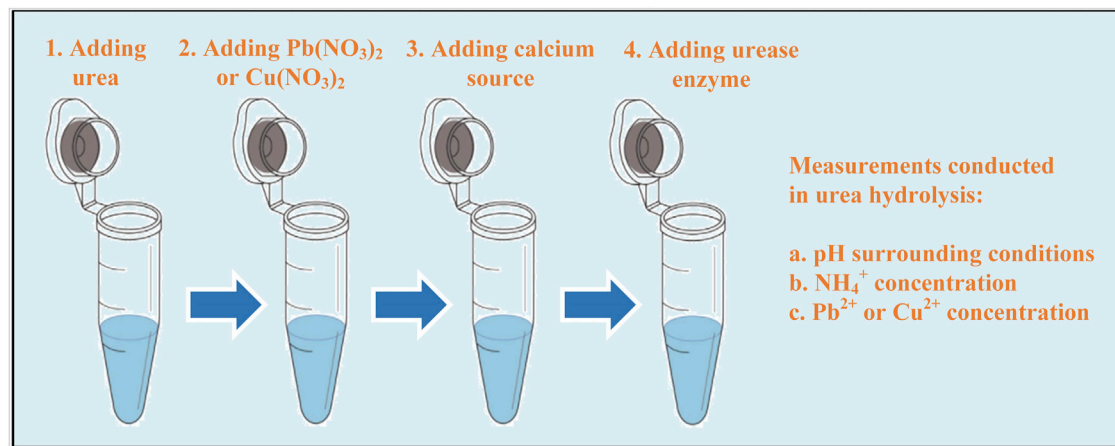


FIGURE 1 | Schematic illustration of the flowchart applied to the test tube experiments.

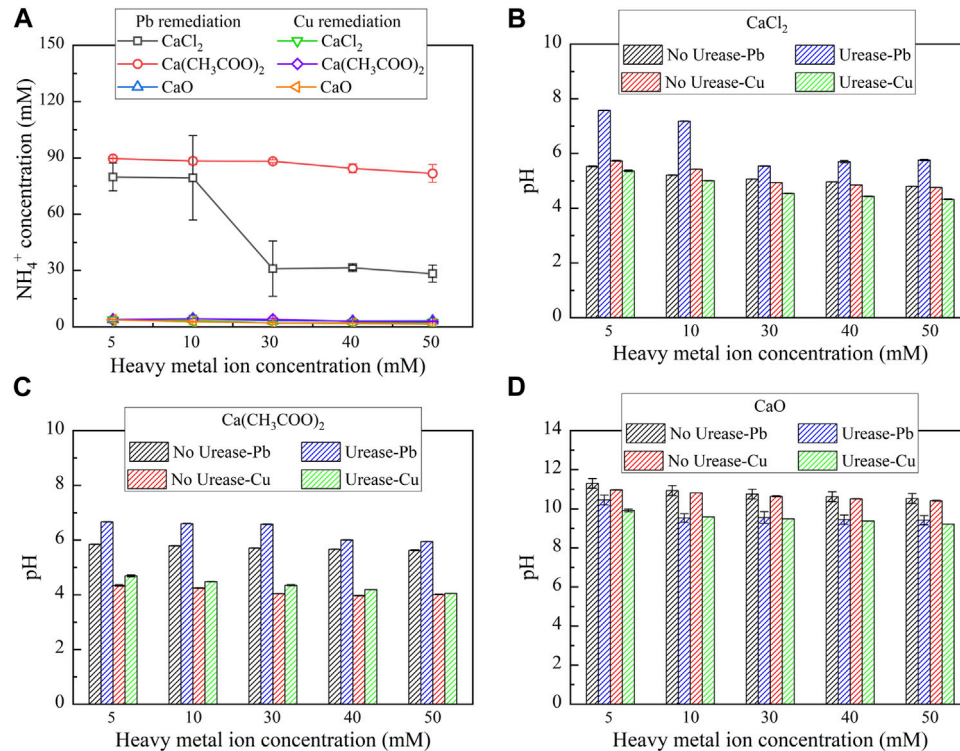
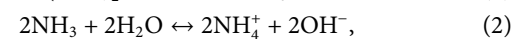
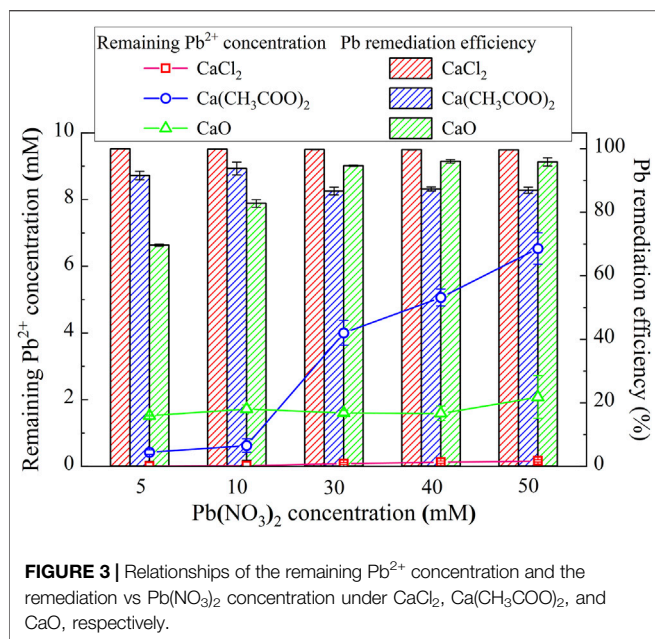


FIGURE 2 | Ammonium ion concentration and pH vs heavy metal concentration: **(A)** NH₄⁺ concentration, **(B)** CaCl₂, **(C)** Ca(CH₃COO)₂, and **(D)** CaO.

et al., 2021b). Notwithstanding that, the application of the EICP method to the remediation of heavy metals is rarely reported. Urease enzyme can be derived from animals and plants. The principle of applying EICP technology to heavy metal remediation is to catalyze urea hydrolysis by the urease enzyme toward discharging ammonia and carbonate ions (NH₄⁺ and CO₃²⁻). By CO₃²⁻ and Ca²⁺, carbonates are formed and precipitated with heavy metal ions (also

referred to as immobilization of heavy metal) where Ca²⁺ ions are derived from the addition of calcium source (Mugwar and Harbottle, 2016). The biochemical reactions that count on urea hydrolysis are shown in **Eqs (1–3)** (Mobley and Hausinger, 1989; Bai et al., 2019; Bai et al., 2020).



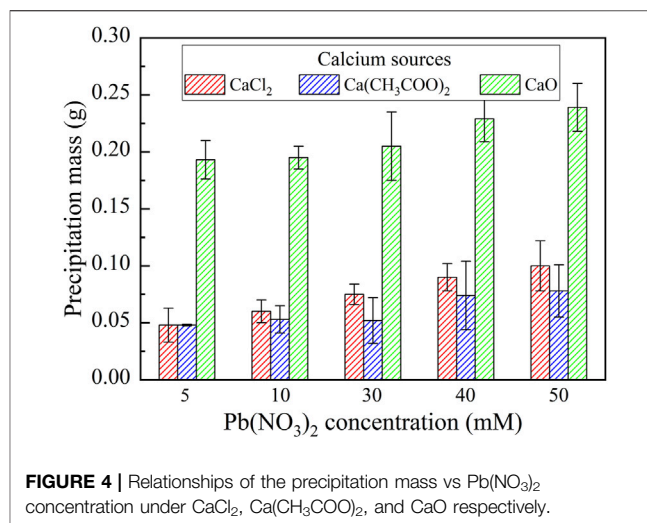


In recent years, EICP technology is also developed as a remediation method for immobilizing inorganic contaminants. Gowthaman et al. (2021) used urease enzyme combined with bone meal to form calcium phosphate biocementation toward decreasing toxic gaseous ammonia by approximately 90%. Furthermore, Moghal et al. (2020) introduced the EICP method that incorporates citric acid (C₆H₈O₇) and ethylene diamine tetra-acetic acid (EDTA) to study the desorption response of heavy metal ions to the soil. Although the literature immobilizes contaminants by EICP technology solely or by a combination of EICP technology with other additions, studies on the mechanisms affecting the remediation efficiency are remarkably limited (Achal and Mukherjee, 2015; Li et al., 2015; Kang and So, 2016). The objectives of this study are 1) to investigate the response of urease activity to high Pb²⁺ and Cu²⁺ concentrations by performing a series of test tube experiments, 2) to investigate the effect of calcium source on the speciation of carbonate precipitation, and 3) to reveal the mechanisms affecting the remediation efficiency for Pb and Cu removals.

MATERIALS AND METHODS

Urease Extraction

Canavalia ensiformis is a known plant to be rich in urease enzyme (Yuan et al., 2020). In the present work, urease enzyme was extracted from *Canavalia ensiformis*. *Canavalia ensiformis* was first ground using a grinding machine and then sieved using a sieve with 150- μm mesh opening. The solution containing jack



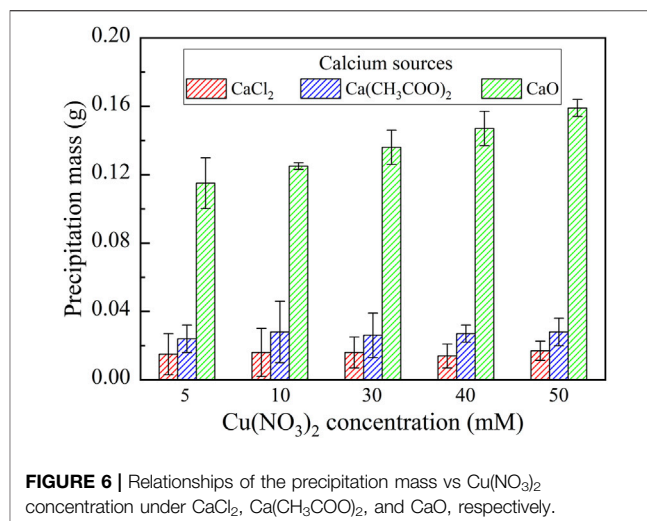
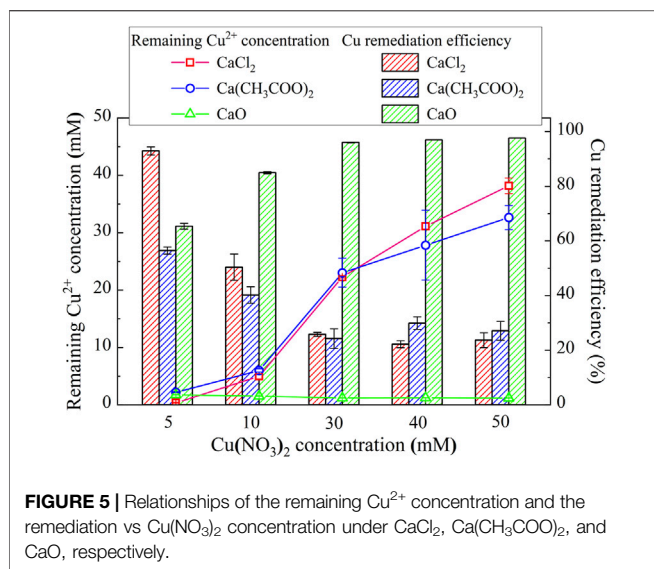
bean and 30% ethanol was centrifuged for 30 min and then stored at 4°C for 4 h. After that, the supernatant was second centrifuged for 1 h and then stored at -20°C for future use.

Urease Activity Measurement

At a temperature of 30°C, 1 international unit (IU) of enzyme activity corresponds to the amount of 1.0 μmol catalyzed transformation in 1 min (Mobley and Hausinger, 1989). In this study, the amount of product (i.e., ammonium ion) catalyzed by urease was measured by Nessler's reagent colorimetric method. Nessler's reagent and potassium sodium tartrate were added, and the absorbance was measured by using a spectrophotometer after waiting for 10 min. The calibration line was set up by the same method prior to the measurement, and the measured absorbance was then substituted to the calibration line to get the concentration of ammonium ions. The urease activity being 342.7 U/g was therefore measured in the present work, which is classed as low activity.

Evaluation of Remediation Efficiency

To investigate the effect of calcium source on copper and lead remediations, a series of test tube experiments were conducted in this study. Three calcium sources, namely, calcium chloride (CaCl₂), calcium acetate (Ca(CH₃COO)₂), and calcium oxide (CaO) were adopted in these test tube experiments to investigate heavy metal (lead nitrate (Pb(NO₃)₂) and copper nitrate (Cu(NO₃)₂)) remediations. A solution applied to the test tube experiments consisted of distilled water, urea, Pb(NO₃)₂ or Cu(NO₃)₂, calcium source, and urease enzyme. The concentration of calcium source used in the test tube experiments was 0.25 M, while the urea concentration was 0.5 M. This concentration range was set to produce enough carbonate to precipitate calcium ions. Heavy metal concentrations adopted included both the low and high values of 5 mM, 10 mM, 30 mM, 40 mM, and 50 mM, respectively. The preparation of the solution is demonstrated in Figure 1.



Measurements of pH and NH₄⁺ concentration, precipitation mass, and remaining heavy metal ion concentration were performed not only to evaluate the urease activity but also for the final assessment of the remediation efficiency. All of the test tube experiments have three replicates. An error bar has already been added to figures where necessary. Statistical analysis indicates that the coefficient of variance for the test tube experiments is below 10%, which is within the requirement being the coefficient of variance of 15% for usual, accessible experimental measurements.

Numerical Simulation

In addition to the test tube experiments, numerical simulations were performed using Visual MINTEQ software package. The numerical simulations, in fact, refer to the concentration of ions (e.g., NH₄⁺ and CO₃²⁻) measured right after the test tube experiments, rather than referring to biochemical processes involved in catalyzing urea hydrolysis. In addition, their stoichiometric ratio sticks to 2:1 (Gat et al., 2017). The concentration of calcium source applied to the simulations was 0.25 M, while NH₄⁺ concentrations (corresponding to the degree of urea hydrolysis) varying in a 10–200 mM range was adopted. The concentration of Pb²⁺ or Cu²⁺ fell in a 5–100 mM range. Given that the degree of urea hydrolysis may affect heavy metal removal, the change in NH₄⁺ concentration benefits us to see how the speciation of carbonate precipitation varies with the degree of urea hydrolysis.

RESULTS AND DISCUSSION

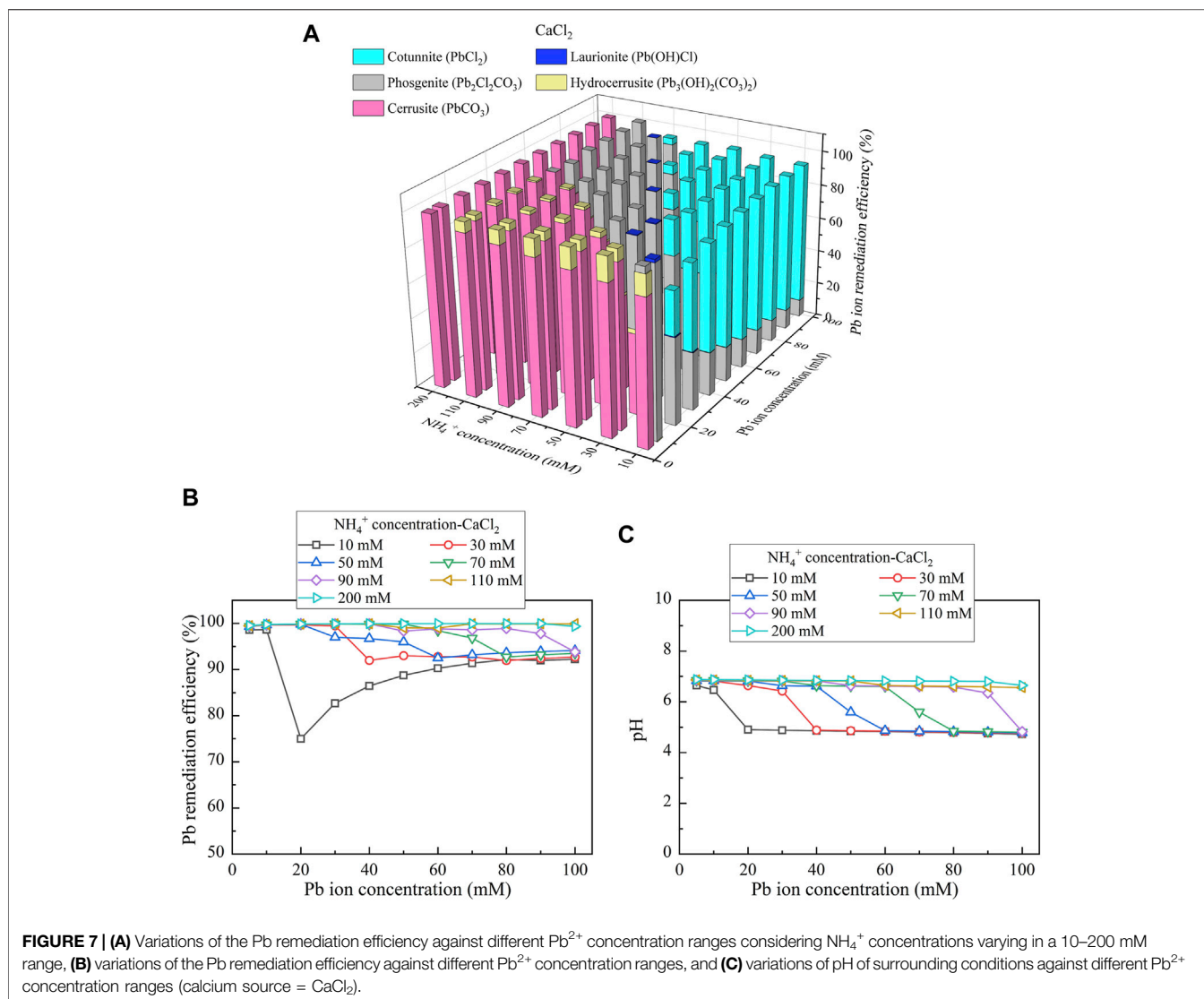
Urea Hydrolysis Response

Urease has been widely applied to catalyzing urea hydrolysis. Ammonium ions (NH₄⁺) and hydroxide ions (OH⁻) are known to be discharged after urea hydrolysis (see Eqs 1–3), and therefore, NH₄⁺ and pH are considered as the key indicators of describing the degree of urea hydrolysis (Fisher et al., 2017; Sun et al., 2021). In

biomineralization, attending higher degrees of urea hydrolysis is considered of great necessity to improve heavy metal remediation efficiency. The relationships of NH₄⁺ and pH vs heavy metal ion concentration under three calcium sources, CaCl₂, Ca(CH₃COO)₂, and CaO, respectively, are shown in Figure 2. Under CaCl₂, NH₄⁺ concentration remaining high (approximately 70 mM) is noted when lower Pb(NO₃)₂ concentrations (i.e., 5–10 mM) are confronted (see Figure 2A). When subjected to higher Pb(NO₃)₂ concentrations (i.e., 30–50 mM), there appears a decline in NH₄⁺ concentration. The higher the concentration of Pb²⁺, the more significant will be the effect on the urease activity and the lower will be the degree of urea hydrolysis. pH remaining in a 7–8 range may be appropriate in securing the urease activity toward elevating the degree of urea hydrolysis (see Figure 2B). The highest NH₄⁺ concentration being about 105 mM is attained under Ca(CH₃COO)₂, corresponding to the highest degree of urea hydrolysis (see Figure 2C). The urease activity, associated with Ca(CH₃COO)₂, remains higher than that under CaCl₂ when pH is falling in the 6–7 range. NH₄⁺ concentration under CaO approaches 0, indicating the absence of urea hydrolysis. Provided there appears remediation efficiency, its attendance relies upon chemical precipitation that is not relevant to the “biomineralization” but to the reaction between Ca²⁺ and OH⁻ (Eq. 4).



Despite that, the chemical precipitation could depress the urease activity by strongly alkaline pH of surrounding conditions, degrading the degree of urea hydrolysis (Mobley and Hausinger, 1989; Schock et al., 1995; Ferris et al., 2004; Hu et al., 2021c) (see Figure 2D). On the other hand, NH₄⁺ concentration under any of the calcium sources always approaches 0 for Cu remediation (see Figure 2A). This is to say that the effect of Cu²⁺ on the urease activity is much higher than the effect of Pb²⁺, thereby reducing the degree of urea hydrolysis. To this end, chemical precipitation is supposed to be present here because of the absence of urea



hydrolysis. pH higher than 9 is observed under CaO, while pH lower than 6 is noted under CaCl₂ and Ca(CH₃COO)₂ (see **Figures 2B–D**). In short, the highest degree of urea hydrolysis presents under Ca(CH₃COO)₂, followed by CaCl₂ and CaO, for the Pb remediation. However, the degree of urea hydrolysis is rather low under any of the calcium sources for the Cu remediation, indicating that there appears remediation efficiency, and chemical precipitation is present here.

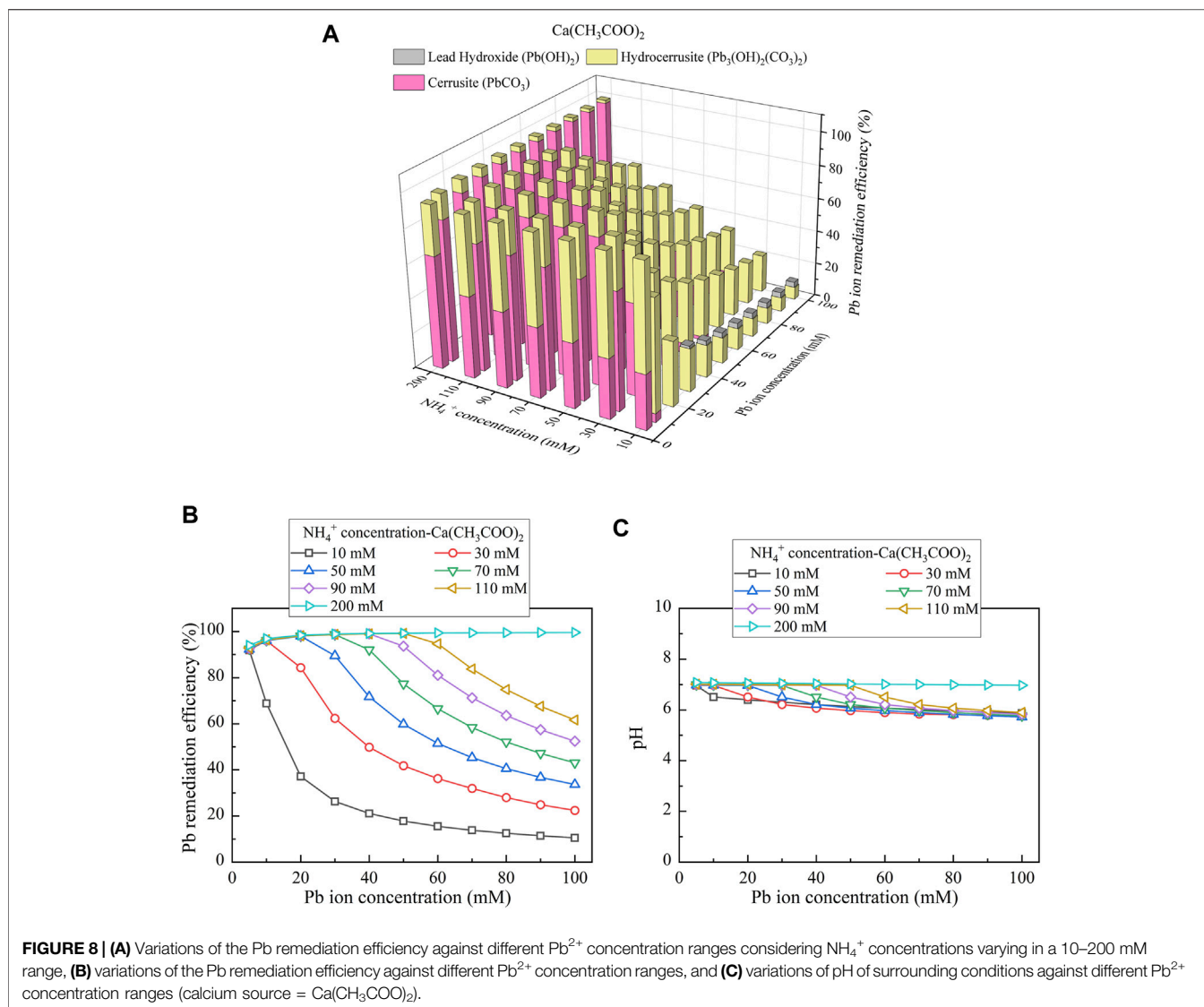
Effect of Calcium Source

This part primarily aims to link the effect of calcium source to the Pb remediation efficiency. The remediation efficiency is defined as follows:

$$\text{Remediation efficiency} = [(C_I - C_R)/C_I] \times 100\%, \quad (5)$$

where C_I represents the initial heavy metal concentration and C_R corresponds to the remaining heavy metal concentration.

The lower the remaining heavy metal ion concentration, the higher will be the remediation efficiency. The relationships of the remaining Pb²⁺ concentration and the remediation efficiency vs Pb(NO₃)₂ concentration under CaCl₂, Ca(CH₃COO)₂, and CaO, respectively, are shown in **Figure 3**. Under CaCl₂, the remediation efficiency higher than 99% (the highest in the present work), with the remaining Pb²⁺ concentration way below 2 mM, is observed, although the precipitation mass is not the highest (see **Figure 4**). It can be inferred that given the second highest degree of urea hydrolysis, biotic precipitation may contribute to the attendance of the highest remediation efficiency. In addition to the biotic precipitation, the highest remediation efficiency could not be achieved without the participation of chemical precipitation. Considering that the chemical precipitation is present earlier than the biotic precipitation, the occupation of Pb²⁺ by the chemical precipitation first depresses the effect of Pb²⁺ on the urease activity, forming



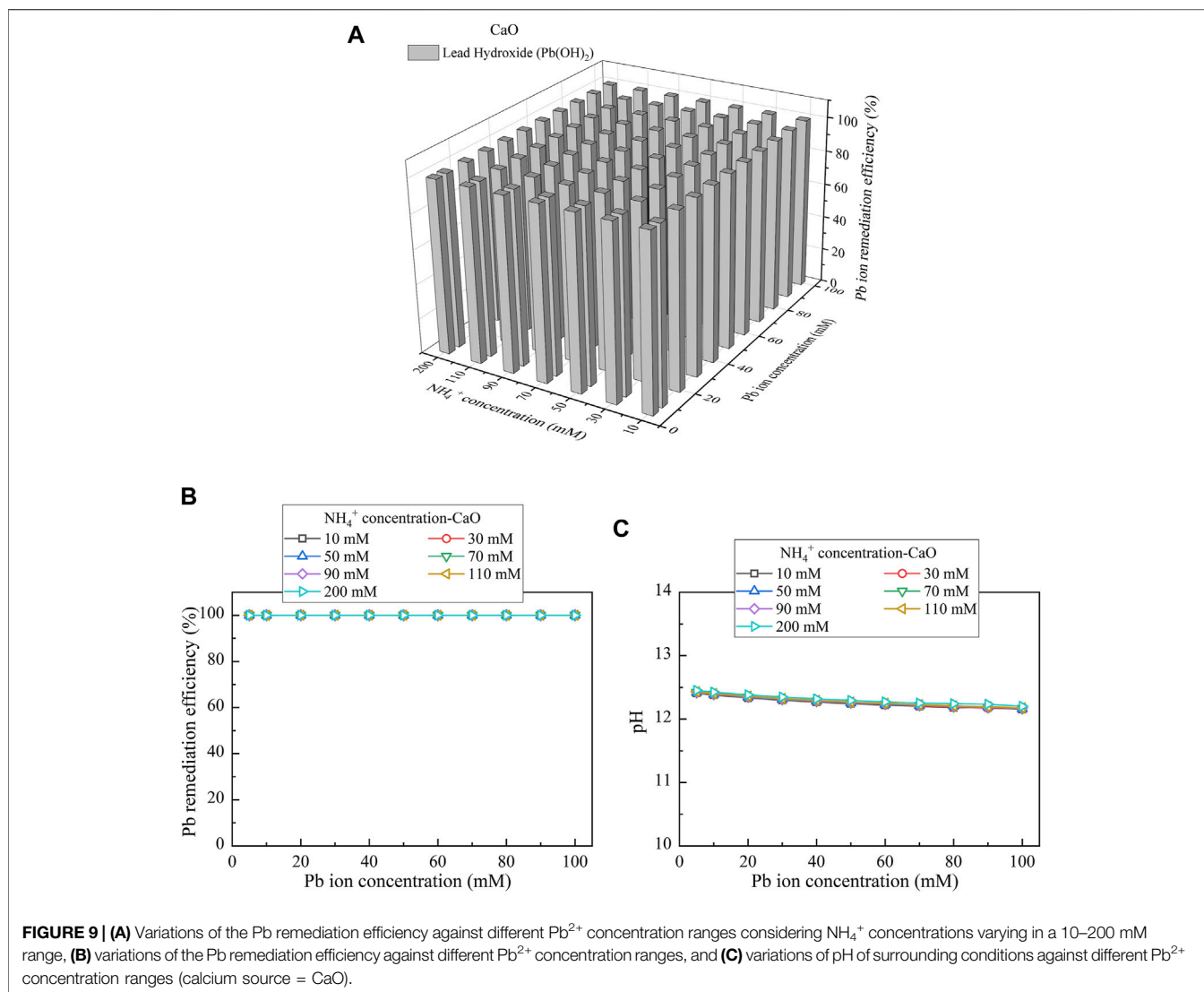
the biotic precipitation by catalyzing urea hydrolysis to assure the highest remediation efficiency.

Under $Ca(CH_3COO)_2$, the degree of urea hydrolysis is the highest, discharging more NH_4^+ and OH^- . The remediation efficiency decreases with the increase in $Pb(NO_3)_2$ concentration. These results indicate that the chemical and biotic precipitations inhibit the effect of Pb^{2+} on the urease activity. However, the depression by the chemical and biotic precipitations turns out to be less significant when subjected to higher $Pb(NO_3)_2$ concentrations. It can also be observed from **Figure 4** that the precipitation mass under $Ca(CH_3COO)_2$ can be compared to that under $CaCl_2$ and is much below that under CaO . On the other hand, under CaO , the degree of urea hydrolysis is the lowest among the three calcium sources; therefore, urea hydrolysis and biotic precipitation that appear are not present here. For this reason, the chemical precipitation,

$Ca(OH)_2$, presents. $Ca(OH)_2$ due to its higher solubility product then transfers to $Pb(OH)_2$.



As discussed, strongly alkaline conditions (higher than 10) could depress urease activity and also raise the dissolution potential of chemical precipitation (see **Figure 2D**). This addresses the degradation in the remediation efficiency when subjected to lower $Pb(NO_3)_2$ concentrations. The role of Pb^{2+} by the chemical precipitations is not only to reduce their effect on the urease activity but also to become more significant when subjected to higher $Pb(NO_3)_2$ concentrations. The remediation efficiency increases with the increase in $Pb(NO_3)_2$ concentration, with the highest measured at about 95%. On the whole, for the Pb remediation, the highest remediation efficiency is attained using $CaCl_2$,



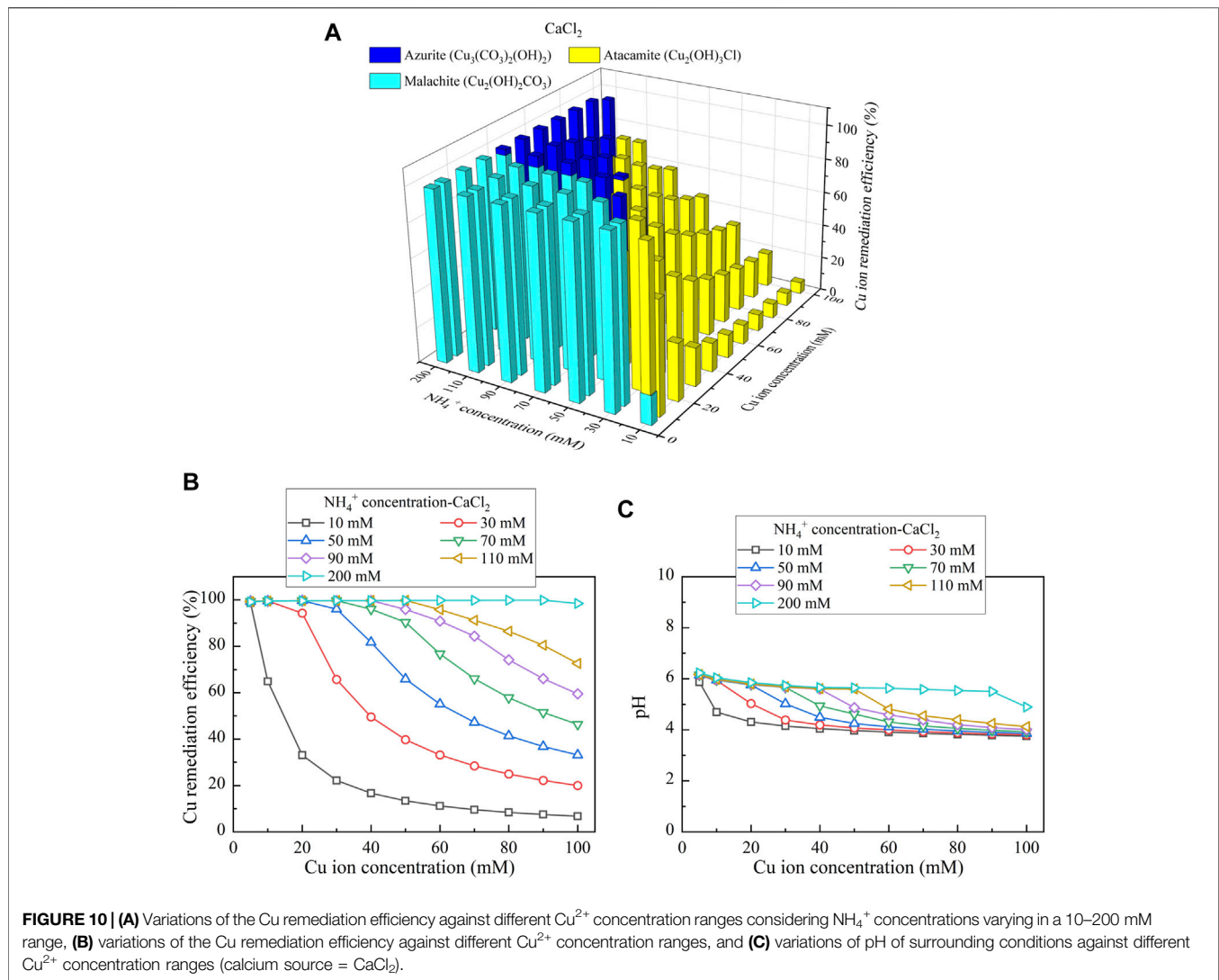
whereas the highest precipitation mass is attained using CaO. Furthermore, the Pb remediation efficiency decreases and increases with the increasing $Pb(NO_3)_2$ concentration under $Ca(CH_3COO)_2$ and CaO, respectively.

This part primarily targets to enhance our knowledge linkage between the effect of calcium source and Cu remediation. The relationships of the remaining Cu^{2+} concentration and the remediation efficiency vs $Cu(NO_3)_2$ concentration under $CaCl_2$, $Ca(CH_3COO)_2$, and CaO, respectively are shown in **Figure 5**. NH_4^+ concentration is much below 15 mM under any of the calcium sources, which corresponds to a very low degree of urea hydrolysis (see **Figure 2A**). Despite that, the highest remediation efficiency is attained using CaO, followed by $CaCl_2$ and $Ca(CH_3COO)_2$, most likely because of the formation of chemical precipitation (Shaw and Raval, 1961). The remediation efficiency beyond 90% is attained using $CaCl_2$ when subjected to $Cu(NO_3)_2$ concentration at 5 mM, and it decreases with the increase in $Cu(NO_3)_2$ concentration. In contrast, under CaO, the remediation efficiency increases with the increasing $Cu(NO_3)_2$ concentration, with

the maximum when subjected to $Cu(NO_3)_2$ concentration varying in a 30–50 mM range. On the other hand, when subjected to $Cu(NO_3)_2$ concentration at 5 mM, the remediation efficiency of up to 55% is attained using $Ca(CH_3COO)_2$, and it decreases with the increase in $Cu(NO_3)_2$ concentration. Similarly, the highest precipitation mass is still attained using CaO, followed by $Ca(CH_3COO)_2$ and $CaCl_2$ (see **Figure 6**). On the whole, the effect of Cu^{2+} badly causes urease to lose its activity. This leads to the inability of catalyzing urea hydrolysis to form biotic precipitation. The highest remediation efficiency is attained using CaO. Considering the main cause leading to the difference in remediation efficiency under $CaCl_2$, $Ca(CH_3COO)_2$, and CaO remains unclear, and it would be discussed later in this article.

Effect of Precipitation Speciation Pb Removal

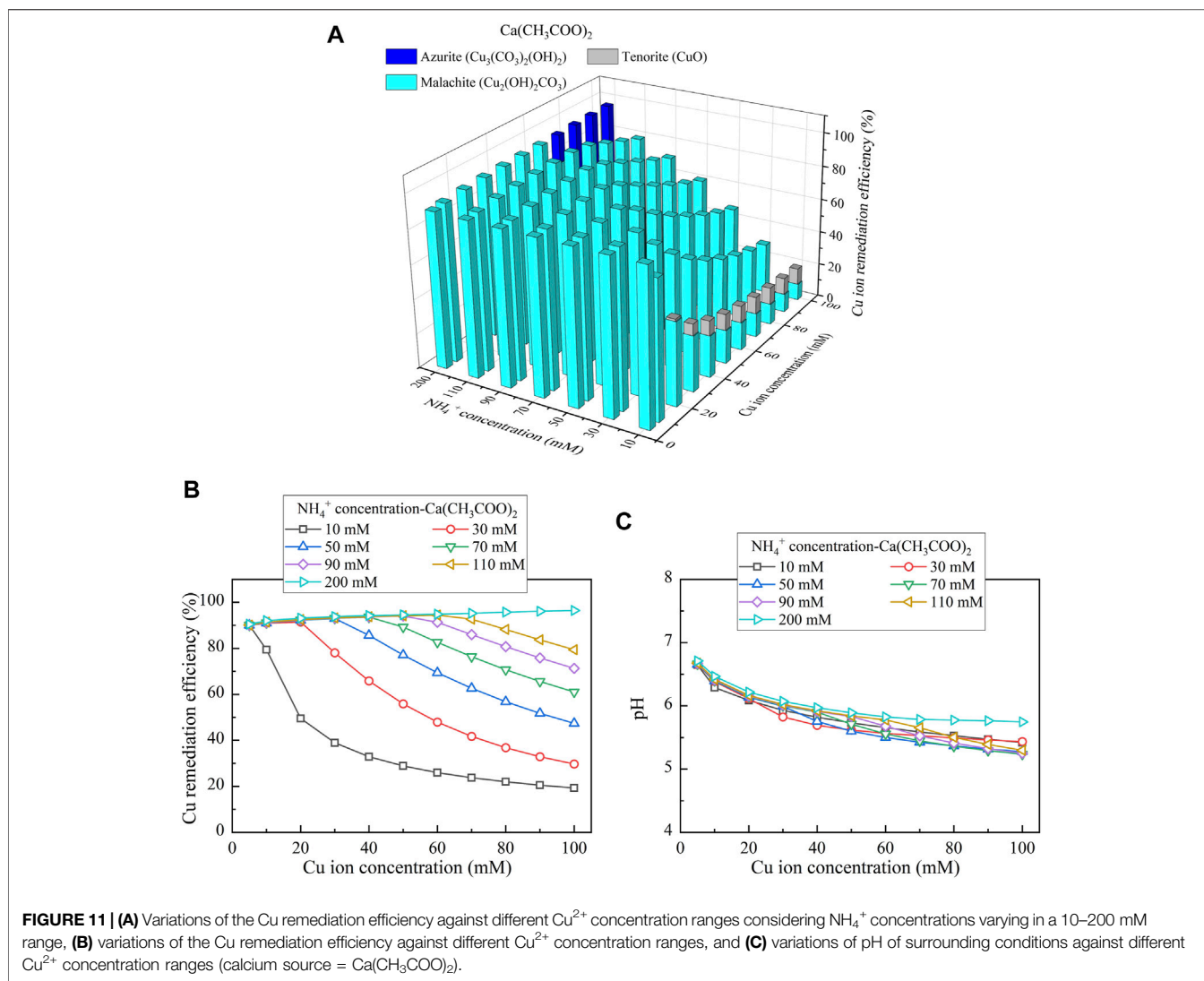
To reveal the speciation of precipitation, a series of simulations were conducted using Visual MINTEQ software package considering different degrees of urea hydrolysis (i.e., NH_4^+



concentration) derived from the test tube experiments. The three calcium sources applied to the experiments also participated in the simulations. The simulated results would give the speciation of precipitation and also benefit to revealing the mechanisms affecting the remediation efficiency. Considering CaCl_2 as the calcium source, the relationships of the remediation efficiency vs NH_4^+ concentration and $\text{Pb}(\text{NO}_3)_2$ concentration are depicted in **Figure 7A**. There are five speciations, including cotunnite (PbCl_2), laurionite ($\text{Pb}(\text{OH})\text{Cl}$), phosgenite ($\text{Pb}_2\text{Cl}_2\text{CO}_3$), hydrocerussite ($\text{Pb}_3(\text{OH})_2(\text{CO}_3)_2$), and cerussite (PbCO_3). Considering the discharge of CO_3^{2-} and NH_4^+ only presents when there appears urea hydrolysis, associated precipitation is also referred to as the “biotic” precipitation. In contrast, precipitation not relevant to CO_3^{2-} is termed as the “chemical” precipitation. To this end, the later three precipitations are categorized as the biotic precipitations, whereas the first two are classified as the chemical precipitations. PbCO_3 , $\text{Pb}_3(\text{OH})_2(\text{CO}_3)_2$, and $\text{Pb}_2\text{Cl}_2\text{CO}_3$ present when the degree of urea hydrolysis is high enough to precipitate the majority of Pb^{2+} .

In total, 1 mM CO_3^{2-} for PbCO_3 is needed to thoroughly precipitate 1 mM Pb^{2+} , whereas for $\text{Pb}_3(\text{OH})_2(\text{CO}_3)_2$, 2 mM CO_3^{2-} is required to precipitate 3 mM Pb^{2+} . When the degree of urea hydrolysis is low and cannot discharge enough CO_3^{2-} and NH_4^+ , $\text{Pb}(\text{OH})\text{Cl}$ and PbCl_2 present. The chemical precipitation in some cases may not be as stable as the biotic precipitation because of its higher solubility product toward causing some difficulty in capsulizing heavy metal ions and raising the concern of their migration. This would eventually degrade the remediation efficiency (Edwards et al., 1992; Baltpurvins et al., 1996).

Figures 7B,C respectively show the variations in the remediation efficiency and pH against different Pb^{2+} concentration ranges considering NH_4^+ concentrations (i.e., degree of urea hydrolysis) varying in a 10–200 mM range. The remediation efficiency of approximately 100% (corresponding to 100 mM Pb^{2+}) is attained using 200 mM NH_4^+ (see **Figure 7B**). In contrast, there appears a substantial reduction in the remediation efficiency to 75% (corresponding to

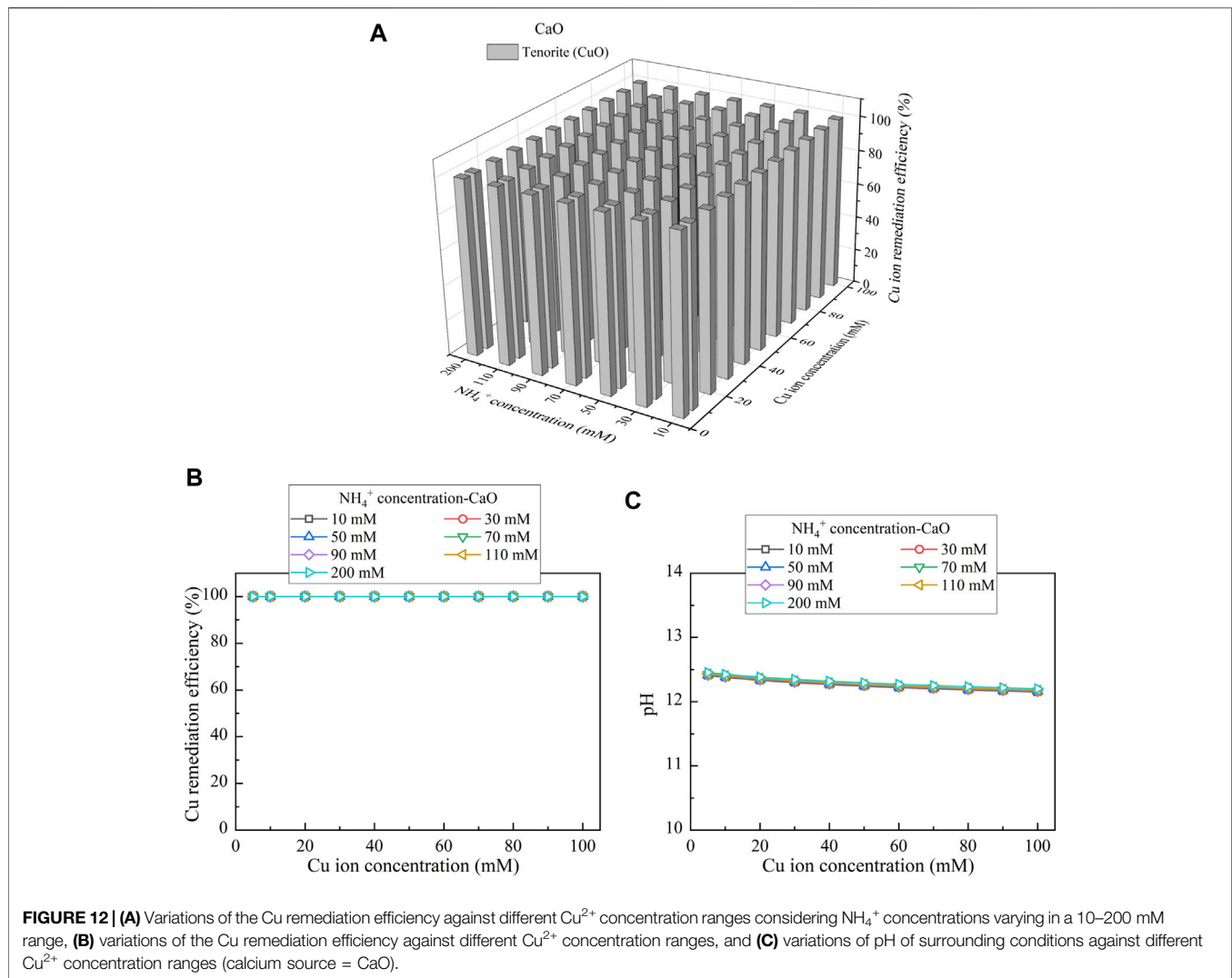


15 mM Pb^{2+}) when Pb^{2+} and NH_4^+ concentrations are 20 and 10 mM, respectively. In total, 10 mM NH_4^+ corresponds to 5 mM CO_3^{2-} , and 5 mM CO_3^{2-} can only precipitate 10 mM Pb^{2+} , in accordance with the speciation of $\text{Pb}_2\text{Cl}_2\text{CO}_3$ (see **Figure 7A**). The remaining 5 mM Pb^{2+} is therefore precipitated by the chemical precipitation (i.e., PbCl_2). The degradation in the remediation efficiency is due to the lack of CO_3^{2-} and NH_4^+ . There appears the other reduction to 90% (corresponding to 36 mM Pb^{2+}) when Pb^{2+} and NH_4^+ concentrations are 40 and 30 mM, respectively. In total, 30 mM NH_4^+ corresponds to 15 mM CO_3^{2-} . As a result, 15 mM CO_3^{2-} can only precipitate 30 mM Pb^{2+} , producing the biotic precipitation (i.e., $\text{Pb}_2\text{Cl}_2\text{CO}_3$) (see **Figure 7A**). The chemical precipitation (i.e., PbCl_2) is to precipitate the remaining 6 mM Pb^{2+} . It is noteworthy that the degradations in the remediation efficiency take place when pH of surrounding conditions become below 6 (see **Figure 7C**). These results indicate that improving the remediation efficiency relies not only on the degree of urea hydrolysis but also on the pH of surrounding conditions (Krajewska, 2016). pH falling in a 6–9

range causes urease to possess hydrolytic activity (Krajewska, 2016). These also satisfactorily address the reduction in the degree of urea hydrolysis when subjected to Pb^{2+} concentrations higher than 30 mM and pH below 6 (see **Figures 2A,B**).

Considering $\text{Ca}(\text{CH}_3\text{COO})_2$ as the calcium source, the relationships of the remediation efficiency vs NH_4^+ concentration and $\text{Pb}(\text{NO}_3)_2$ concentration are shown in **Figure 8A**. There are three speciations of precipitation, including cerussite (PbCO_3), hydrocerussite ($\text{Pb}_3(\text{OH})_2(\text{CO}_3)_2$), and lead hydroxide ($\text{Pb}(\text{OH})_2$), where the last is classified as the chemical precipitation, and the other two are classified as the biotic precipitation. The remediation efficiency of approximately 100% (corresponding to 100 mM Pb^{2+}) is attained via 200 mM NH_4^+ (see **Figure 8B**). Similarly, there appears a reduction in the remediation efficiency when CO_3^{2-} and NH_4^+ concentrations are not high enough. The degradations in the remediation efficiency initiate when pH stays away from 7 (see **Figure 8C**). They develop further when pH drops consistently.

When CaO is considered as the calcium source, the relationships of the remediation efficiency vs NH_4^+



concentration and $\text{Pb}(\text{NO}_3)_2$ concentration are shown in **Figure 9A**. The precipitation corresponds to one single speciation, namely, $\text{Pb}(\text{OH})_2$. $\text{Pb}(\text{OH})_2$ is categorized as the chemical precipitation. The remediation efficiency as high as 100% is attained irrespective of NH_4^+ concentration, indicating the absence of urea hydrolysis (see **Figure 9B**). As discussed, $\text{Ca}(\text{OH})_2$ precipitates earlier than $\text{Pb}(\text{OH})_2$, and the former turns pH into strongly alkaline conditions, causing the dissolution of precipitation (see **Figure 9C**). The dissolution of precipitation, in turn, degrades the remediation efficiency when subjected to lower $\text{Pb}(\text{NO}_3)_2$ concentrations (see **Figure 3**). pH turns however into weakly alkaline conditions when subjected to higher $\text{Pb}(\text{NO}_3)_2$ concentrations. This tackles difficulty in catalyzing urea hydrolysis under strongly alkaline conditions and therefore raises the remediation efficiency. The experimental results appear to conflict with the simulated ones, most likely due to the neglect of precipitation dissolution by the simulations. The remediation efficiency remaining very high under pH above

12 provides testimony of the aforesaid argument (see **Figure 9C**).

Cu Removal

This part presents the simulated results applied to the Cu removal. Considering CaCl_2 as the calcium source, the relationships of the Cu remediation efficiency vs NH_4^+ concentration and $\text{Cu}(\text{NO}_3)_2$ concentration are shown in **Figure 10A**. Three speciations of precipitation are identified. Azurite ($\text{Cu}_3(\text{CO}_3)_2(\text{OH})_2$) and malachite ($\text{Cu}_2(\text{OH})_2\text{CO}_3$) are classified as the biotic precipitations, while atacamite ($\text{Cu}_2(\text{OH})_3\text{Cl}$) is classified as the chemical precipitation. Furthermore, under $\text{Ca}(\text{CH}_3\text{COO})_2$, $\text{Cu}_3(\text{CO}_3)_2(\text{OH})_2$, tenorite (CuO), and $\text{Cu}_2(\text{OH})_2\text{CO}_3$ are recognized, as shown in **Figure 11A**. Moreover, using CaO as calcium source, single one speciation, namely, CuO, is identified, as shown in **Figure 12A**. It can be seen from **Figures 10B,C**, **11B,C** that under CaCl_2 and $\text{Ca}(\text{CH}_3\text{COO})_2$, the lack of CO_3^{2-} and NH_4^+ triggers a degradation in the remediation efficiency. In addition to the

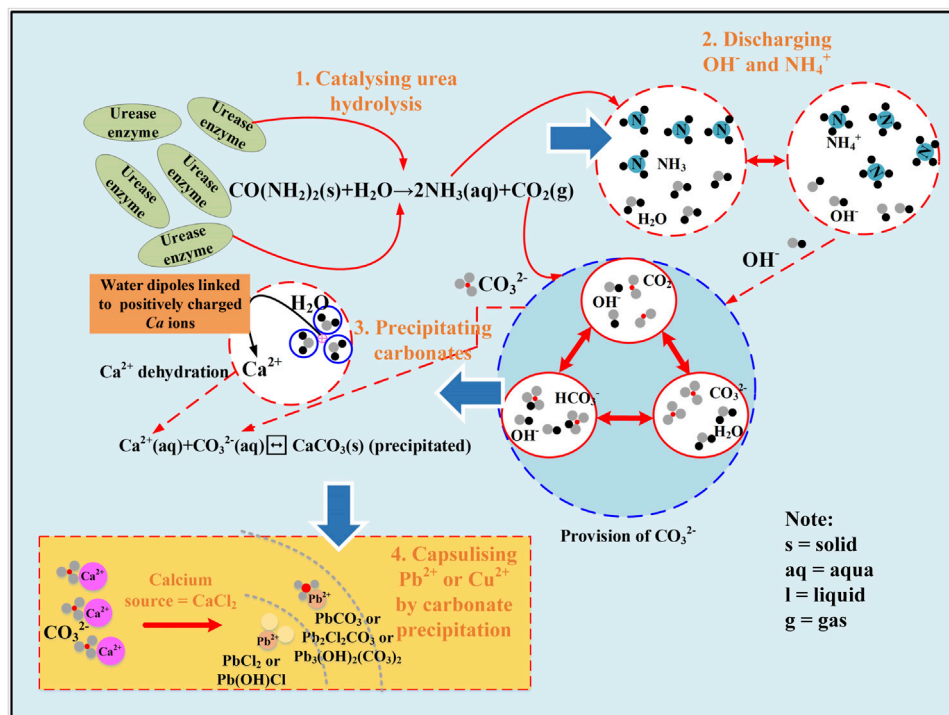


FIGURE 13 | Schematic illustration of the mechanisms affecting the remediation efficiency.

lack of CO_3^{2-} and NH_4^+ , the degradation in the remediation efficiency is also counted on the remediation efficiency. Under CaO, it dissolves in water to form $\text{Ca}(\text{OH})_2$, and $\text{Ca}(\text{OH})_2$ turns pH into highly alkaline conditions toward affecting the urease activity and eventually degrading the remediation efficiency (see Figures 12B,C). The degradation in the remediation efficiency is however absent here as the numerical simulation neglects the precipitation dissolution. To summarize, the effect of Cu^{2+} can more significantly reduce urease activity. The highest remediation efficiency is attained using CaO. Such high remediation efficiency still presents when subjected to lower $\text{Cu}(\text{NO}_3)_2$ concentrations, which is not in line with the experimental results. The neglect of precipitation dissolution by the simulations is deemed as the main cause leading to the discrepancy. The remediation efficiency degrades when CO_3^{2-} and NH_4^+ concentrations are not high enough to precipitate the majority of Cu^{2+} . In addition, the remediation efficiency also degrades when pH turns into highly alkaline conditions, induced by the addition of CaO.

Mechanisms Affecting Remediation Efficiency

Heavy metal ions can significantly affect the urease activity and cause some difficulty in catalyzing urea hydrolysis and capsulizing heavy metal ions toward degrading the remediation efficiency. Urease can be capsulized by the addition of calcium sources, preventing loss of their

activity. Under CaCl_2 and $\text{Ca}(\text{CH}_3\text{COO})_2$, NH_4^+ concentration for the Pb remediation remains as a high provide testimony of the aforesaid argument. As a result, the biotic precipitation can precipitate the majority of Pb^{2+} toward securing the remediation efficiency. However, under CaO, NH_4^+ concentration is approximately 5 mM, which also indicates the absence of urea hydrolysis. In this case, the remediation efficiency relies upon the chemical precipitation. The degradation in the remediation efficiency presents when subjected to lower $\text{Pb}(\text{NO}_3)_2$ concentrations. $\text{Ca}(\text{OH})_2$ turning pH into highly alkaline conditions is considered as the leading cause resulting in the degradation of the remediation efficiency. Therefore, the degradation in the remediation efficiency not only relies on the provision of NH_4^+ and OH^- but also on the change in pH, induced by the addition of calcium source. NH_4^+ concentration for the Cu remediation is measured as low as approximately 5 mM, irrespective of calcium source. As a result, the remediation efficiency is attained not only by the biotic precipitation but also by the chemical precipitation. It is worth noting that under CaO, the reduction as appeared when subjected to lower $\text{Cu}(\text{NO}_3)_2$ concentrations in the test tube experiments is absent in the simulated results. The discrepancy is mainly attributed to the neglect of precipitation dissolution under highly alkaline conditions. To conclude, ensuring the degree of urea hydrolysis is deemed of great necessity when applying the enzyme-induced carbonate precipitation technology to heavy metal remediation. There are two leading causes affecting the

remediation efficiency: one is the provision of NH_4^+ and OH^- , and the other is the change in pH, resulting from the addition of calcium source (see **Figure 13**). Here, NH_4^+ and OH^- concentrations high enough can precipitate the majority of Pb^{2+} and Cu^{2+} , corresponding to the biotic precipitation. When NH_4^+ and OH^- concentrations are not high enough to precipitate the majority of Pb^{2+} and Cu^{2+} , the remediation efficiency is attained through a combination of biotic and chemical precipitations. On the other hand, CaO dissolves in water to produce $\text{Ca}(\text{OH})_2$ toward turning pH into highly alkaline conditions. Such alkaline conditions could not only depress the urease activity but also cause dissolution of the carbonate precipitation, thereby degrading the remediation efficiency. The degradation is absent in the simulated results because the simulations do not take the dissolution of carbonate precipitation into account.

CONCLUSION

The test tube experiments were conducted to reproduce catalyzing urea hydrolysis using urease enzyme. The experimental and numerical results were applied to investigate the effect of calcium source on Pb and Cu removals. Based on the results and discussions, some main conclusions can be drawn as follows:

- 1) The remediation efficiency degrades when NH_4^+ and OH^- concentrations are not sufficient to precipitate the majority of Pb^{2+} and Cu^{2+} . The remediation efficiency due to the lack of Pb^{2+} and Cu^{2+} is attained through a combination of the biotic and chemical precipitations.
- 2) Highly alkaline conditions could not only affect the urease activity but also cause dissolution of carbonate precipitation.

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CaO dissolves in water to produce $\text{Ca}(\text{OH})_2$, turning pH more alkaline, and degrading the remediation efficiency.

- 3) The numerical simulations are deemed useful in enhancing our knowledge linkage between the speciation of carbonate precipitation and the remediation efficiency and preventing unfavorable pH of surrounding conditions from affecting the degree of urea hydrolysis. Notwithstanding that, the simulations do not take the dissolution of precipitation into account and therefore overestimate the remediation efficiency when subjected to lower $\text{Pb}(\text{NO}_3)_2$ or $\text{Cu}(\text{NO}_3)_2$ concentrations.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding author.

AUTHOR CONTRIBUTIONS

LW: data curation, formal analysis, validation, software, and writing—original draft. W-CC: conceptualization, methodology, writing—review and editing, supervision, and funding acquisition. Z-FX: writing—review and editing.

FUNDING

This paper is based upon work supported by the Shaanxi Educational Department under Grant No. 2020TD-005 through the innovative ability support scheme.

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