



Research Paper

Rhamnolipid stabilized nano-chlorapatite: Synthesis and enhancement effect on Pb- and Cd-immobilization in polluted sediment



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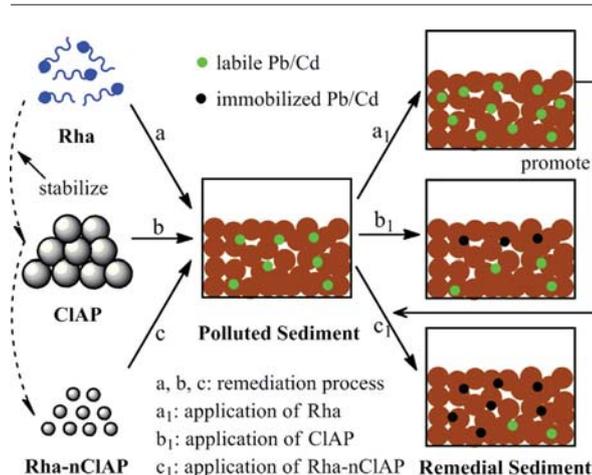
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HIGHLIGHTS

- Rha-nCIAP was synthesized with the combination of Rha and CIAP.
- Rha-nCIAP could effectively immobilize Pb and Cd and reduce the TCLP-leachable Pb and Cd.
- Rha could enhance the Pb and Cd immobilization by promoting the metal liberation from sediment.
- The organic matter and available phosphorus indicated the microorganism may involve.

GRAPHICAL ABSTRACT



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ABSTRACT

Phosphate (P) compounds are usually used as chemical amendment for *in situ* remediation of heavy metal polluted sediment. However, the low deliverability, weak utilization and potential risk of eutrophication inhibit the application of most P materials. Therefore, rhamnolipid (Rha), a kind of anionic biosurfactant which has algicidal activity, was employed in this study to synthesize a new kind of nano-chlorapatite (nCIAP) for Pb and Cd immobilization. Characterization results showed that the Rha stabilized nCIAP (Rha-nCIAP) was uniformly distributed in suspensions within about 5 nm. Experimental data demonstrated that the combination of Rha and nCIAP could greatly enhance the Pb- and Cd-immobilization efficiencies, promoting their transformation from labile fractions to stable fractions through precipitation or adsorption processes, especially when the Rha approached to its critical micelle concentration. And Rha-nCIAP could also decrease both the TCLP-leachable Pb and Cd with maximum reduction efficiencies of 98.12% and 96.24%, respectively, which also presented concentration dependence

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of Rha. Changes of available phosphorus implied the dissolution of nCIAP during the treatment and the detection of organic matter demonstrated that the microorganisms may involve in the remediation.

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1. Introduction

Lead (Pb) and cadmium (Cd) are widespread contaminants in sediments and soils [1–3]. They usually came into the environment through metal smelting, waste discarding, rubbish incineration and the application of agrochemicals [3–6]. Sediment polluted with Pb and Cd is of great concern to the government and researchers since they could accumulate in human body through the uptake by plants and other organisms [7–11]. Pb and Cd can not be degraded through microbial or chemical processes and they could persist in sediment for a long time, posing a potential toxicity to organisms [12–18]. It is necessary to find an effective method to deal with the polluted sediment since heavy metals in sediment are the intermediate sources for the occurrence of heavy metal pollution in aquatic ecosystem [19,20]. Current technologies for heavy metal remediation in sediment are divided into *in situ* and *ex situ* methods. Generally, the *in situ* method aims to remove the polluted sediments for the further treatment or dispose it while the *in situ* method figures on preventing the toxic metals from accessing to the aquatic ecosystem by capping an inert material or transferring the metals into a stable fraction with the using of various amendments [21,22].

Phosphate (P) amendments, including soluble phosphate, insoluble phosphate and other synthesized P-based materials, are of great importance for heavy metal remediation since P-materials could cooperate well with many heavy metals such as Cd(II), Pb(II), Cu(II), and Zn(II) and convert these metals from unstable fraction to stable fraction, reducing their toxicity and bioavailability [23–27]. Phosphates of Pb [such as $Pb_5(PO_4)_3F$, $K_{sp} 10^{-71.6}$; $Pb_5(PO_4)_3Cl$, $K_{sp} 10^{-84.4}$; $Pb_5(PO_4)_3Br$, $K_{sp} 10^{-78.1}$; $Pb_5(PO_4)_3(OH)$, $K_{sp} 10^{-76.8}$] and Cd [such as $Cd_5(PO_4)_3(OH)$, $K_{sp} 10^{-42.49}$; $Cd_5(PO_4)_3Cl$, $K_{sp} 10^{-49.66}$] were much less soluble than other Pb and Cd compounds [28,29]. According to BCR (European Community Bureau of Reference) sequential extraction method, these metal-phosphate compounds are often recognized as the residual fraction (F-res) [other fractions are: acid soluble/exchangeable fraction (F-exc); reducible fraction (F-red) and oxidizable fraction (F-oxi), the stability of them are in order of: F-exc < F-red < F-oxi < F-res] which is insoluble and almost unreactive under most pH and oxidative conditions [30–32]. Therefore, more and more researchers pay their attention on the P-based materials for *in situ* remediation and results showed that P was effective to immobilize Pb and Cd in soils and eliminate their bioavailability [1,33–37]. Previous studies showed that the immobilization efficiency of P mostly depends on the amount of available phosphorus in contaminated sediments and soils since the soluble P performed much better than the insoluble P during the remediation [25,38,39]. However, the most intractable problem associated with P application appears to be the water eutrophication induced by the leaching of excess P, especially in case of soluble P [28]. In order to enhance the immobilization efficiency and decrease the eutrophication risk of P-materials, researchers tried to modify the insoluble P-material (in which the P is much less bioavailable to the algae than soluble P) with different substrates (such as carboxymethyl cellulose, sodium dodecyl sulfate and biochar) to get nano-sized, well-dispersed and easily-delivered P particles in recent years [1,2,35,40].

Rhamnolipid (Rha), a kind of environmental friendly anionic biosurfactant which produced by *Pseudomonas aeruginosa* and has algicidal activity on the harmful algal bloom species was employed

in this study to synthesize a new kind of stabilized nano-chlorapatite (nCIAP) for Pb and Cd remediation in polluted sediment [41]. In light of the problems associated with P application, this paper hypothesizes that the combination of rhamnolipid and chlorapatite would enhance the (Pb and Cd) immobilization efficiency of chlorapatite and at the same time decrease the eutrophication risk induced by P. To investigate the Pb and Cd immobilization efficiency of synthesized nano-chlorapatite and understand the immobilization mechanism involved, the BCR sequential extraction method, toxicity characteristic leaching procedure (TCLP), available phosphorus and organic matter are measured during the remediation.

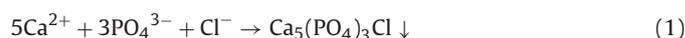
2. Materials and methods

2.1. Materials

All the chemicals used in this study were of analytical or higher grade. Rhamnolipid (Rha, the mixture of Rha-Rha-C₁₀-C₁₀ and Rha-C₁₀-C₁₀) was purchased from Huzhou Zijin Biological Technology company. The ultrapure water (18.25 MΩ cm, 25 °C) was prepared by an Ultra-pure Water System and all the equipments were washed extensively with the ultrapure water. The experiment was conducted in triplicate and the results were expressed as mean ± standard deviation.

2.2. Preparation and characterization of Rha-nCIAP

Rhamnolipid stabilized chlorapatite (Rha-nCIAP) was synthesized according to the steps proposed by Liu and Zhao [1] with some modifications: a 30 (50 and 60) mg/L Rha solution, a 26.8 mM Ca²⁺ and a 16.0 mM PO₄³⁻ solution were prepared by dissolving Rha, CaCl₂·2H₂O and Na₃PO₄·2H₂O into the ultrapure water accurately, respectively. The pH of Rha solution was adjusted to 8.40 by using an appropriate amount of 50 mM NaOH solution. Then 25 mL of 26.8 mM Ca²⁺ was dropped slowly (5–8 drops per minute) into 50 mL 30 (50 and 60) mg/L Rha solution by the acid burette under the constantly mixing (magnetic stirrer with a Teflon-coated stir bar) at 1000 r/min. After constantly stirring for 12 h, another 25 mL of 16.0 mM PO₄³⁻ solution was added to the mixture under constantly mixing with 1500 r/min at 5–8 drops per minute. To give a completely blending of Rha and CIAP, the mixture was continued stirred for 12 h at 1500 r/min after the addition of PO₄³⁻. A control experiment that used the ultrapure water (pH = 8.40) instead of Rha solution was prepared with other conditions unchanged. Fig. 1 is the laboratory flowchart about the synthesis of Rha-nCIAP. Concentrations of Rha were chosen on the base of its critical micelle concentration (CMC, about 25 mg/L) which exerts great impact on the properties of Rha. The molar ratio of Ca²⁺ to PO₄³⁻ was 1.67 in respect to the chemical formula of CIAP [Ca₅(PO₄)₃Cl] according to Liu and Zhao [1]:



To investigate the particle distribution of synthesized Rha-nCIAP and observe its cross section structure, a drop of Rha-nCIAP suspension was placed on a carbon-coated copper grid and kept at 28 °C for 24 h for transmission electron microscopy (TEM, JEM-3010, JEOL, Japan) analysis. Then the Rha-nCIAP suspension was freeze-dried by a freeze-dryer (LGJ-10B, Beijing sihuan scientific

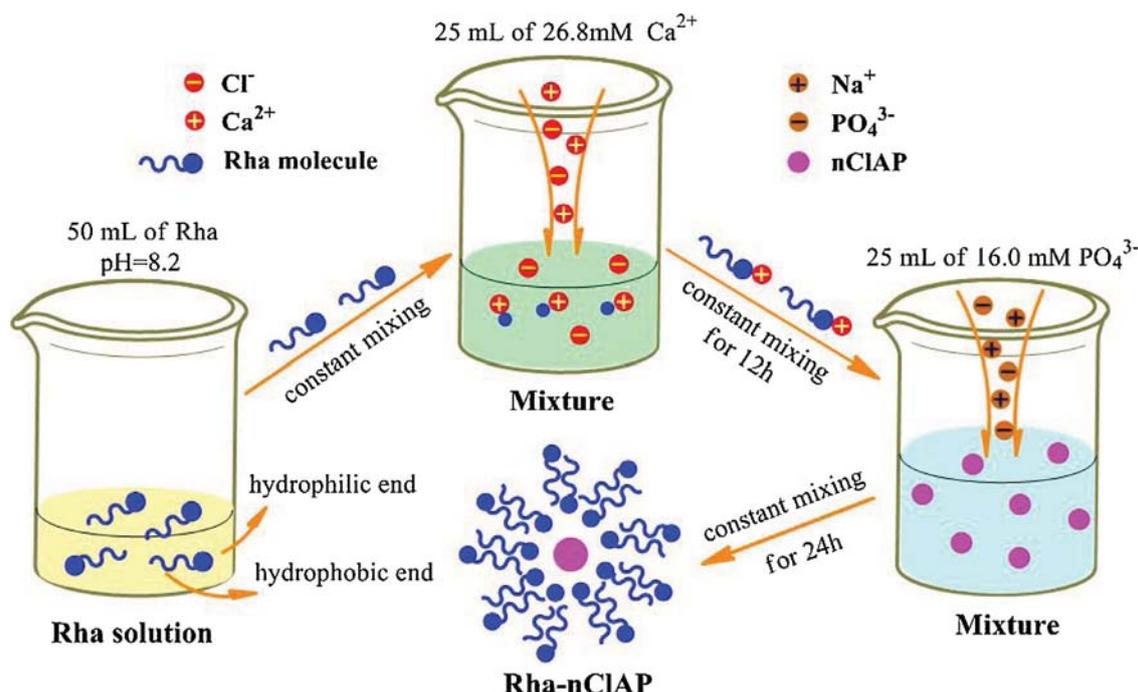


Fig. 1. The laboratory flowchart about the synthesis of Rha-nCIAP.

instrument factory co., LTD, China) to get the Rha-nCIAP powder for further analysis. Field emission scanning electron microscope (FESEM, JSM-6700, JEOL, Japan) was taken after placed the Rha-nCIAP powder onto a piece of double coated carbon conductive tape and sprayed by gold for 60 s. The energy dispersive X-ray (EDAX) was conducted by the EDAX attachment on FESEM to analyze the elementary composition of Rha-nCIAP. To analyze the functional groups on the surface of Rha-nCIAP, Fourier transform infrared spectrometry (FTIR) was taken on a Shimadzu FTIR spectrophotometer (IRAffinity-1) using KBr pellet method at spectral range varying from 4000 to 400 cm^{-1} . The zeta potential of Rha solution at pH of 8.40 was measured by a Zeta potential analyzer (Zetasizer Nano zs90).

2.3. Pretreatment of sediment samples

The sediment samples collected from Xiawangang River (Zhuzhou, Hunan province in China) were tilled on the cleaned containers and dried naturally for 5–7 d. To remove large fragments, gravel and organic debris, we sieved the samples through a 2 mm nylon sieve. Then a pestle and mortar were used to ground them extensively and further sieved the grounded sediments to $150 \mu\text{m}$. At last, the prepared sediment samples were stored at 4 °C and kept dry before use. The sediment was slightly alkaline (pH = 7.98) with high organic matter (5.89%) and had a high cation exchange capacity (48.7 cmol/kg). The concentrations of Pb and Cd in the sediment were about 589.7 and 309.5 mg/kg, respectively, both of which greatly exceeded the national standard (350 mg/kg for Pb and 0.6 mg/kg for Cd, respectively) [2].

2.4. Sediment treatment with Rha-nCIAP

0.5 g (2.5 g for the analysis of available phosphorus) prepared sediment were placed into a 50 mL circled plastic centrifuge tube and the Rha-nCIAP suspension was also added into the centrifuge tube at a suspension-to-soil ratio of 10:1 (mL/g). The control experiment was taken with chlorapatite suspension instead and other conditions were unchanged. To investigate the possible effects of

Rha on Pb/Cd immobilization, a series of Rha solution (15, 25 and 30 mg/L) were also added with the same ratio. The treated samples are named separately according to the different amendment materials as follows: A: Rha-nCIAP within 15 mg/L Rha; B: Rha-nCIAP within 25 mg/L Rha; C: Rha-nCIAP within 30 mg/L Rha; D: 15 mg/L Rha; E: 25 mg/L Rha; F: 30 mg/L Rha; G: CIAP. All the mixture samples were kept at 25 °C in the room conditions. These samples were treated from 1 to 45 d before sampling at 4000 r/m for 20 min, then the supernatant and sediment were stored separately for further analysis.

2.5. Measurement of TCLP

The TCLP-leachable Pb and Cd were measured according to the procedure described in our previous study [2]. The stored supernatant was measured by flame atomic absorption spectrometry (AAS700, PerkinElmer, USA).

2.6. BCR extraction

To measure changes of the chemical speciation of Pb and Cd in treated sediment, the BCR experiment used in our previous study [2] was conducted in this study. The recovery rates of Pb and Cd in this experiment were ranging from 93% to 108%, which were consistent with other studies [42,43].

2.7. Available phosphorus (AP) and organic matter (OM)

To determine the AP and OM in the samples, the methods recommended by Bray and Kurtz [44] and Nelson and Sommers [45] were used with some modifications, respectively. AP was detected by the molybdenum-antimony anti-spectrophotometric method and the OM was measured by dilution heating method.

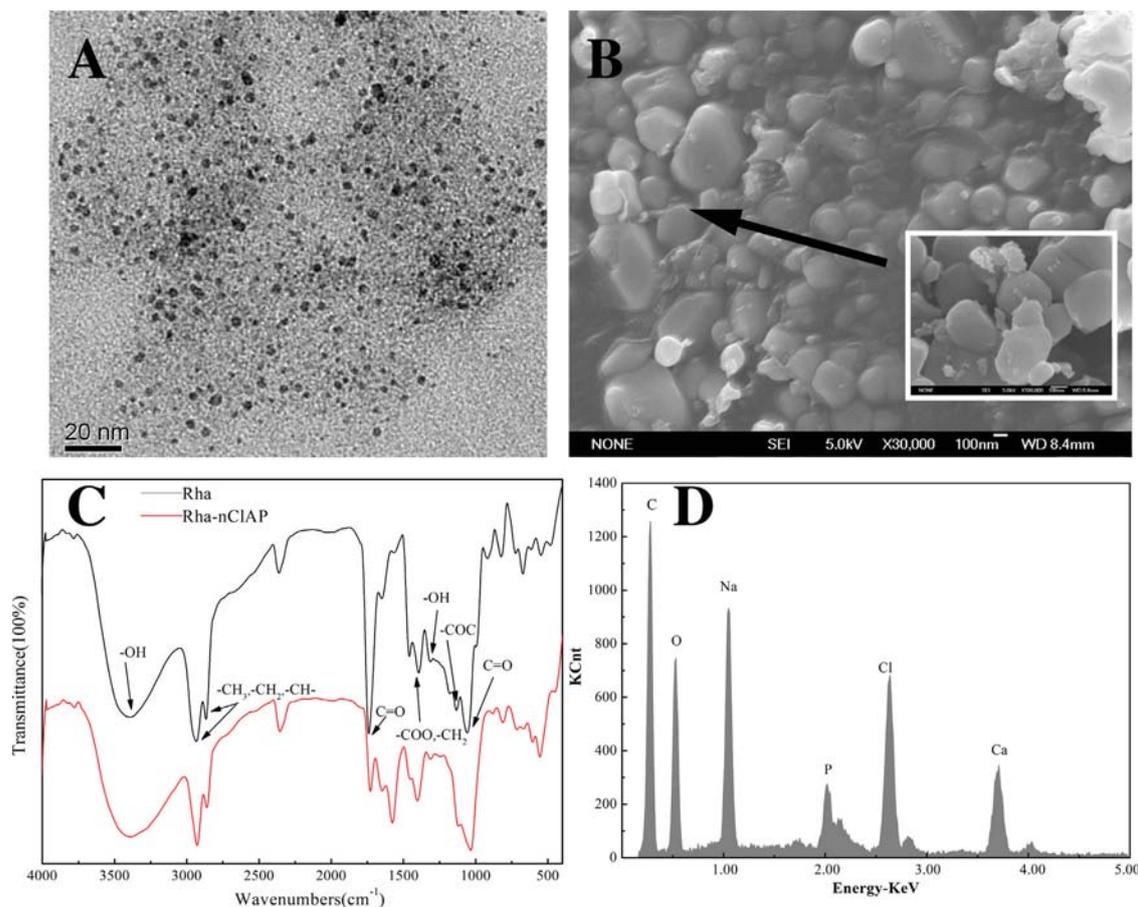


Fig. 2. Characterization of the synthesized Rha-nClAP within 25 mg/L Rha: (A) TEM image of Rha-nClAP. (B) FESEM image of Rha-nClAP. (C) FTIR spectra of Rha-nClAP and pure Rha in the region between 4000 and 400 cm^{-1} , respectively. (D) EDAX analysis of the Rha-nClAP.

3. Results and discussions

3.1. Characterization of Rha-nClAP

Fig. 2 provides a general characterization of the synthesized Rha-nClAP. From the TEM results showed in Fig. 2A, we can see that the average diameter of these Rha-nClAP particles was estimated at approximately 5 nm with a uniform distribution. A FESEM microphotograph presented in Fig. 2B was to further analyze the morphology of the nanoparticles. It can be concluded from the FESEM image that the synthesized Rha-nClAP particles were in a spherical or spheroidicity shape. The average diameter of Rha-nClAP particles in FESEM image (ranging from 10 to 100 nm) was much bigger than that in TEM image, which may attribute to the aggregation of the particles during the freeze-drying process since only a small drop of the nanoparticle suspension was needed for TEM image. On the other hand, previous studies also demonstrated that the macromolecules such as carboxy methylated cellulose and sodium dodecyl sulfonate were invisible under TEM, which may induce the over-estimated of the particle size in SEM image [2,46].

Fig. 2D was the EDAX spectrum of the Rha-nClAP particles. The obvious peaks showed in Fig. 2D were related to the elements of C, O, Na, P, Cl and Ca, in which the Ca, P, O, Cl were the main components of chlorapatite while C, O were the dominate elements in Rha (H can not be detected by EDAX). The Ca/P ratio shown in the EDAX results approximated to the ratio of Ca/P in $\text{Ca}_5(\text{PO}_4)_3\text{Cl}$, demonstrating that the products were chlorapatite. Liu and Zhao [1], who synthesized the chlorapatite using the same method, confirmed that the synthesized particles were chlorapatite according to the analysis of the XRD patterns. Additionally, from the FTIR spectrums

of Rha and Rha-nClAP shown in Fig. 2C we can see that the dominating function groups in Rha (such as $-\text{OH}$, $-\text{CH}_2$ and $\text{C}=\text{O}$) were also found in the Rha-nClAP with the same location. That is to say, the chlorapatite had been successfully synthesized and stabilized by Rha.

3.2. Effects of Rha-nClAP on the immobilization of Pb

Fig. 3 showed the changes of Pb chemical speciation and TCLP-leachable Pb fraction in each treated sample during 0–45 d. The original data about Pb chemical speciation presented in Fig. 3a showed that the F-exc, F-red, F-oxi and F-res occupied 8.2%, 11.1%, 60.5% and 20.2% in untreated sample, respectively. The original TCLP-leachable Pb in our sediment was 0.30 mg/L, which posed direct threat to organisms. In our study, the Rha treated samples (sample E, D and F) without any addition of ClAP showed no effects on the F-oxi and F-res fraction in sediment during 45 d while the F-exc was gradually increased with the decrease of F-red. On the other hand, the F-res of Pb in Rha-nClAP treated samples (sample A, B and C) were increased during the treatment, especially in sample B with 25 mg/L (the CMC of Rha) Rha in Rha-nClAP. Thus we can see that the sample B in which the Rha-nClAP was synthesized by using 25 mg/L Rha showed the biggest increase of F-res (40%), followed by sample A (28%) and sample C (27%). Simultaneously, as shown in Fig. 3b, the TCLP-leachable Pb fraction also exhibited the same tendency: Rha-nClAP within 25 mg/L Rha was the most effective material to decrease the TCLP-leachable Pb in polluted sediment when compared with other materials. And, along with the increase of F-exc in Rha treated samples (D, E, F), the TCLP-leachable Pb were also increased in these samples, especially in

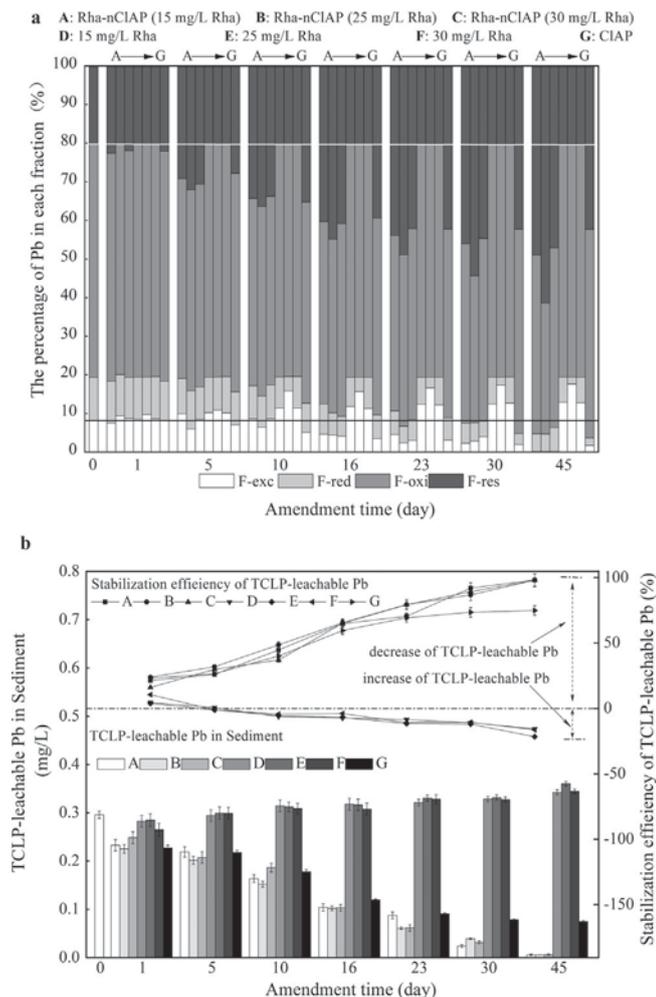
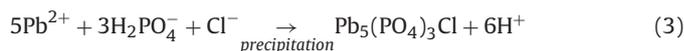
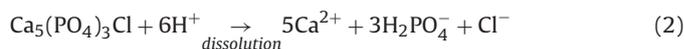


Fig. 3. The changes of Pb chemical speciation (a) and TCLP-leachable Pb fraction (b) in Rha-nCIAP, Rha and CIAP treated samples, respectively. Day 0 represents the original data of untreated sample. The error bars represent the standard deviation of the means ($n = 3$).

sample E (25 mg/L Rha). However, the sample G which was treated by CIAP without any addition of Rha, showed an obvious increase in F-res with a nonnegligible decrease in F-exc and F-red, and the TCLP-leachable Pb in sample G was also decreased. Even though the CIAP could immobilize the labile Pb and decrease the TCLP-leachable Pb in sediment, Pb immobilization efficiencies in sample A, B and C were apparently higher than sample G.

According to the study of Mulligan et al. [47], the biosurfactants such as Rha could result in the detachment of the heavy metal from the soil into the soil solution but has no effect on the residual fraction, which was in agreement with our study. Additionally, it also has been proved in previous studies that CIAP was effective to immobilize Pb in sediment and its immobilization efficiency could be enhanced by modifications such as sodium dodecyl sulfate (SDS) and carboxyl methyl cellulose (CMC₁) [1,2]. Therefore, owing to the metal-detachment effect of Rha [which could provide assistance for Pb to release from F-exc and F-red (Fig. 3a)], the Rha-nCIAP showed a higher ability to transfer Pb from unstable fraction (F-exc and F-red) to stable fraction (F-res) when compared with SDS-nCIAP and CMC₁-nCIAP [1,2], reducing its toxicity to organisms. From the results in Fig. 3a we can see that even the Rha-nCIAP treated samples (A, B and C) showed more advantages in Pb immobilization than CIAP and Rha treated samples, the concentration of Rha in Rha-nCIAP posed a great impact on Pb-stabilization efficiency and the CMC of Rha was of vital importance during the immobilization.

It has been demonstrated in some studies that the elution effect of metals was greatly influenced by the surfactant concentration: it was increased with the increase of surfactant concentration below CMC while the high concentration above CMC may result in the inhibition of metal removal [48]. This may attribute to the different stabilities of the amendments since the zeta potential of 25 mg/L Rha (CMC of Rha) was -35.2 mV while it were -19.0 and -22.8 mV for 15 mg/L Rha and 30 mg/L Rha, respectively (data not shown). As confirmed by Klitzke et al. [49], the higher zeta potential (surface charge) of the colloids could result in an increasing metal mobilization, which would contribute to Pb immobilization in our study. In our previous study we have proposed the Pb stabilization process by CIAP [Eqs. (2) and (3)]. In combination with the study of Mavropoulos et al. who put forward the dissolution-precipitation process for P-induced Pb removal, it can be concluded that the liberation of Pb from the sediment was usually the limited factor during the Pb immobilization by P-materials [50,51]. Therefore, the remediation effectiveness of Rha-nCIAP mostly depends on the concentration of Rha in it while the Rha exhibited the various elution effects of metals under different concentrations. In conclusion, the Rha-nCIAP was a promising amendment to reduce the Pb toxicity in sediment by promoting the leaching of Pb from sediment and immediately forming stable Pb-complexes, especially when the concentration of Rha approximated to the CMC.



3.3. Effects of Rha-nCIAP on the immobilization of Cd

For the stabilization experiment of Cd, the Rha-nCIAP even presented the same immobilization behaviors (Fig. 4). In Fig. 4a we can see that the Cd chemical speciation in original sediment consisted of: 11.4% of F-exc, 42.4% of F-red, 34.3% of F-oxi and 12.0% of F-res, respectively. As shown in Fig. 4a, the CIAP was effective to transfer Cd from F-exc and F-red to F-res, reducing the Cd toxicity. Moreover, this was more obvious for Rha-nCIAP treated samples (A, B, C) in which F-res were gradually increased during the treatment within 45 d while the F-exc and F-red were decreased rapidly. On the other hand, F-exc in sample D, E and F were increased under the treatment of Rha while the F-res of Cd in these samples were maintained at about 12.0% without any remarkable changes. As to sample B, in which the Rha-nCIAP contained 25 mg/L Rha, the F-res of Cd were increased to 50.6% after 45 d treatment. However, the immobilization advantage of Cd in sample B was not that apparent than the stabilization of Pb since the F-res of Cd in sample A and C could also reach to 47.78% and 44.78% after 45 d treatment, respectively. The change of TCLP-leachable Cd in sediment during the treatment was shown in Fig. 4b. It can be found in Fig. 4b that the original TCLP-leachable Cd of the sediment was about 0.28 mg/L, and it was decreased to 0.01 mg/L (approximately 97% reduction) with the treatment of Rha-nCIAP after 45 d whether in sample B or sample A and C. For the Rha treated sediments, the TCLP-leachable Cd was increased during the treatment, especially in sample E with 25 mg/L Rha. The sediment which was treated by the CIAP exhibited a 41.0% reduction of TCLP-leachable Cd after 45 d. Thus we can see that the combination of Rha and CIAP may enhance the CIAP-induced Cd immobilization, which was the same as the case of Pb stabilization.

In the early study of Xu et al. [52], apatite was confirmed to be an effective agent for Cd²⁺ removal from solutions. They also demonstrated that complexation with functional groups such as $\equiv\text{POH}$ [Eqs. (4) and (5)] and coprecipitation with Ca²⁺ to hydroxyapatite [Eq. (6)] were the main mechanisms for Cd removal. Additionally,

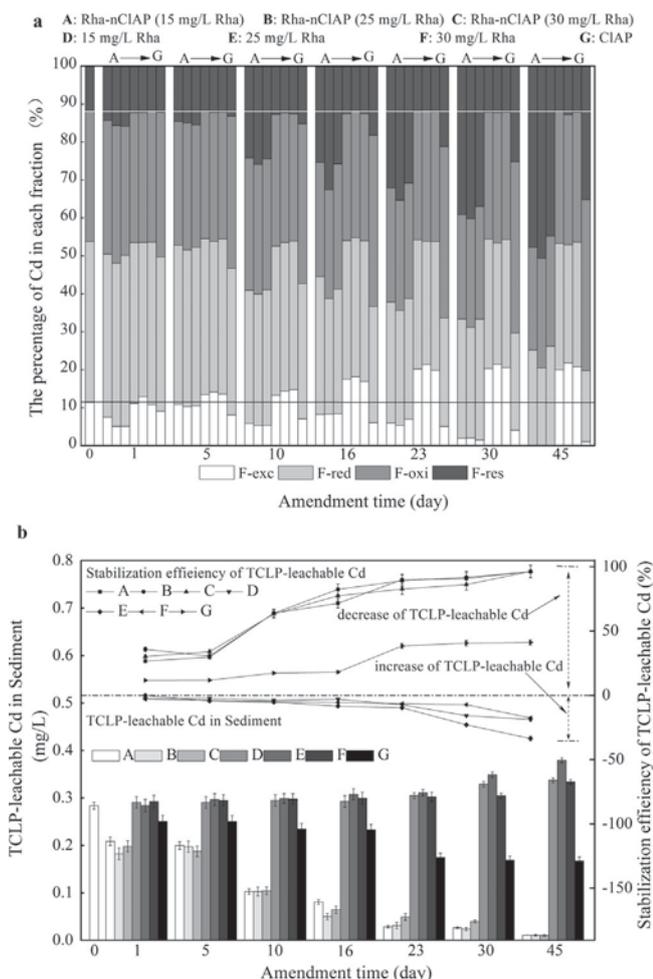


Fig. 4. The changes of Cd chemical speciation (a) and TCLP-leachable Cd fraction (b) in Rha-nCIAP, Rha and CIAP treated samples, respectively. Day 0 represents the original data of untreated sample. The error bars represent the standard deviation of the means (n=3).

other researchers also found that the addition of P could increase the Cd immobilization and reduce the phytotoxicity of Cd in variable charge soils [53]. In case of our study, CIAP was used for Cd stabilization in polluted sediment and results turned out that CIAP could also immobilize Cd by transferring Cd from F-exc and F-red to F-res [such as $Cd_3(PO_4)_2$, $K_{sp} = 10^{-38.1}$; $Cd_5(PO_4)_3Cl$, $K_{sp} = 10^{-49.66}$], thus reducing its toxicity and bioavailability. The main mechanism could be explained by the coprecipitation reaction shown in Eq. (6) proposed by Xu et al. [52]. As we can see in Fig. 4, the Rha-stabilized CIAP was more effective for Cd immobilization than CIAP, which may attribute to the metal-leaching effect of Rha as we mentioned above. The concentration of Rha in Rha-nCIAP not only played an important role for Pb stabilization but also posed a direct effect on Cd immobilization. Results revealed that the TCLP-leachable Cd and F-exc of Cd in sediment were increased more apparently when treated by 25 mg/L Rha. According to the study of Klitzke et al. [49], higher zeta potential of solutions may enhance the metal mobilization by electrostatic stabilization. Therefore, more liable Cd has been observed in sample E which possessed a higher zeta potential (-35.2 mV). Moreover, among the Rha-nCIAP treated samples, the 25 mg/L Rha stabilized nCIAP was more efficient for Cd immobilization since the Rha could provide more liable Cd by elution effect under CMC [48]. Consequently, the Rha stabilized nCIAP combined the advantages of Rha and CIAP during the remediation: the Rha acted as an eluent for metal liberation from sediment while the

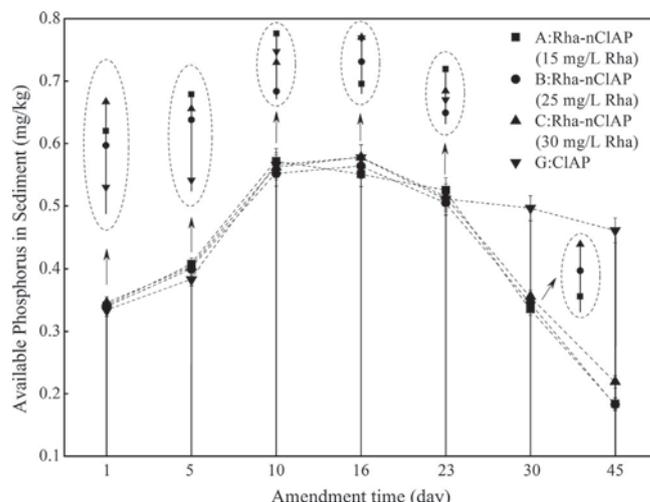
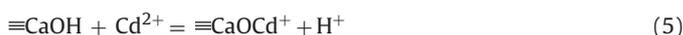


Fig. 5. Available phosphorus in treated samples during 45 d treatment. The error bars represent the standard deviation of the means (n=3).

CIAP played as metal precipitating agent, thus greatly improved the metal stabilization efficiency.



3.4. The changes of available phosphorus: possible mechanism

It has been analyzed in Sections 3.2 and 3.3 that the Rha-nCIAP and CIAP could induce the immobilization of Pb and Cd since the F-res in these samples were increased during the treatment. To our knowledge, the main mechanism for the increase of F-res in P-based materials treated samples was about the formation of metal-phosphate compounds, which were mainly induced by the precipitation or adsorption process and even insoluble under most conditions [28]. For the precipitation of Pb-phosphate and Cd-phosphate, the release of PO_4^{3-} will be one of the main factors that controlling the immobilization efficiency.

Fig. 5 showed the changes of AP in treated samples during the amendment. The original AP level in sediment was 0.01 mg/kg. It can be observed that the AP exhibited a sharply increase (from 0.01 to 0.33 mg/kg) after the addition of Rha-nCIAP and CIAP for 1 d. This increasing trend lasted for about 16 day until it went down at 23rd, and it experienced a remarkable decrease from 23 d to 45 d. The increase of AP at the early stage may be attributed to the dissolution of $Ca_5(PO_4)_3Cl$ as we have presented in Eq. (2). In combination with the phenomena we have observed in Figs. 3 and 4, it also can be concluded that the sharply decrease of AP may be induced by the formation of Pb-phosphates or Cd-phosphates complexes such as $Pb_5(PO_4)_3Cl$ ($K_{sp} 10^{-84.4}$) and $Cd_5(PO_4)_3Cl$ ($K_{sp} 10^{-49.66}$), which was partly in agreement with our previous study [2]. The formation of the insoluble metal-phosphate compounds may occur through the precipitation, coprecipitation and adsorption processes according to the reported researches [1,2,36]. As seen in Fig. 5, the AP levels among sample A, B and C even showed no significant differences during the treatment, which indicated that the Rha concentration has no effect on the dissolution of $Ca_5(PO_4)_3Cl$. Furthermore, it also revealed that the metal-immobilization differences among the Rha-nCIAP treated samples may relate to the different concentrations of Rha in the materials but had no relationships with the CIAP.

Additionally, it can be found in Fig. 5 that the AP levels in Rha-nCIAP treated samples were apparently higher than CIAP treated

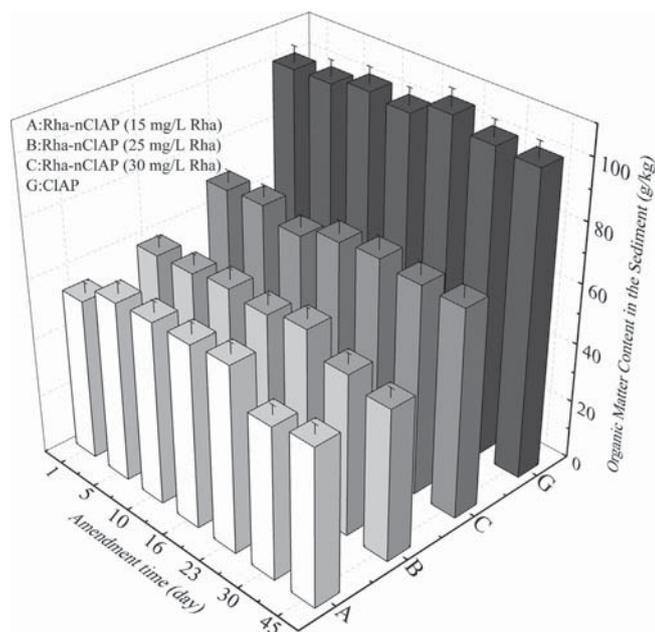


Fig. 6. Organic matters in treated samples during 45 d treatment. The error bars represent the standard deviation of the means ($n = 3$).

samples after 23 d while these samples were almost exhibited the same AP level before 23 d, and the AP in CIAP treated sample at 45 d was almost above 0.45 mg/kg. This result may relate to the big size and low deliverability of CIAP which inhibited the delivery of P and decreased its utilization efficiency, leading to a mediocre result of metal-stabilization. On the other hand, the excessive AP resulted from the dissolution of P-material could become a potential threat to the organisms since P would induce the eutrophication [24,29]. Moreover, the Rha has been proved to be effective in destroying the growth of harmful algal bloom species. Consequently, the application of Rha stabilized CIAP was more environmental friendly than the CIAP.

3.5. The effects on organic matters

In our study, the OM changes in Rha-nCIAP and CIAP treated samples during the amendment have been investigated and results were shown in Fig. 6. It can be found in Fig. 6 that the OM content in CIAP treated sample was approximately twice than Rha-nCIAP treated samples during the treatment. That may attribute to the present of Rha in Rha-nCIAP since Rha was a kind of anionic biosurfactant secreted by *Pseudomonas aeruginosa* and had some relationships with the metabolism of microbes. And the OM contents in these samples was in the order of: $C > B > A$, which may result from the different Rha contents in Rha-nCIAP materials. What's more, the OM experienced a slight increase at first then decreased at 23rd in sample A, B and C during the treatment, and the final OM contents in treated samples were changed when compared with the original data in untreated sediment (58.94 g/kg, data not shown). However, this phenomenon has also been found in the change of AP during the treatment (Section 3.4). In a recent study reported by Zhu et al. [54], the γ -polyglutamic acid (γ -PGA, artificial compounds) could enhance the PR (phosphate rock)-induced Pb immobilization efficiency and inhibit the Pb accumulation in plants while the only addition of γ -PGA or PR cannot do that. Moreover, studies also indicated that fungus could accelerate the dissolution of insoluble P materials by secreting organic acids and promote the formation of metal-phosphate [55]. Additionally, Wang et al. [56] investigated the influence of organic matter on P-induced Pb immo-

bilization in contaminated soil, they found that the addition of humic acid may promote the transformation of stabilized products from $PbHPO_4$ to the more insoluble products such as $Pb_5(PO_4)_3(OH)$ and $Pb_5(PO_4)_3Cl$, which were confirmed by SEM, TEM, X-ray diffraction and laser scanning confocal microscope. Therefore, the organic matters, either secreted by microorganisms or presented by other ways, play an important role in altering the occurrence of heavy metal. That was to say, the enhanced metal immobilization efficiency of Rha-nCIAP may be probably induced by Rha through influencing OM contents and AP levels in sediments during the treatment. To our knowledge, the microbe and its behaviors may be the agent between P amendments and OM, and it should be investigated more extensively in further studies to give a better understanding of the P-induced Pb and Cd immobilization.

4. Conclusions

Rhamnolipid stabilized nCIAP was successfully synthesized for Pb and Cd immobilization in polluted sediment in this study. The synthesized products were characterized by TEM, FESEM, FTIR and EDAX, and results showed that the particles were uniformly distributed in suspensions within about 5 nm. Remediation experiment demonstrated that Rha-nCIAP could transfer the metal from labile fraction (F-exc, F-red and F-oxi) to stable fraction (F-res) more effectively through precipitation or adsorption processes than CIAP. Furthermore, we also found that the Rha concentration in synthesized Rha-nCIAP could greatly influence the immobilization efficiency and it may relate to the surface charge of the amendments since higher zeta potential may enhance the metal mobilization by electrostatic stabilization. When the Rha concentration in Rha-nCIAP approximated to CMC, either the stabilization efficiency of Pb or the Cd was the best. Thus we have concluded that the Rha acted as an eluent for metal liberation from sediment while the CIAP played as metal precipitating agent, thus greatly improved the metal stabilization efficiency. On the other hand, from the analysis about the change of AP we found that the CIAP could be used more efficiently by the metal after being modified by Rha, which could also decrease the potential risk of eutrophication. More importantly, the Rha was confirmed to be effective in preventing the growth of harmful algal bloom species, indicating that the application of Rha-nCIAP was more environmental friendly than the CIAP. The OM showed that the Rha may have some inner relationships with the behaviors of microbes, indicating that the effect on micro-organisms should also be taken into consideration in our further study.

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