



Combined modification of clay with sulfhydryl and iron: Toxicity alleviation in Cr-contaminated soils for mustard (*Brassica juncea*) growth



Yingheng Fei^b, Chengshuai Liu^{a,c,*}, Fangbai Li^c, Manjia Chen^c, Hui Tong^c, Chuanping Liu^c, Changzhong Liao^c

^a State Key Laboratory of Environmental Geochemistry, Institute of Geochemistry, Chinese Academy of Sciences, Guiyang 550081, China

^b Guangzhou Institute of Advanced Technology, Chinese Academy of Sciences, Guangzhou 511458, China

^c Guangdong Key Laboratory of Agricultural Environment Pollution Integrated Control, Guangdong Institute of Eco-Environment and Soil Sciences, Guangzhou 510650, China

ARTICLE INFO

Article history:

Received 30 June 2015

Revised 25 October 2016

Accepted 25 October 2016

Available online 27 October 2016

Keywords:

Bentonite

Chromium

Soil remediation

Thiol modification

Thiol/Fe modification

ABSTRACT

Clays are promising adsorbents for heavy metal removal from the environment because they are economical, naturally abundant and environmentally stable. Application of natural clays, however, are typically limited due to their adsorption capacity and because they usually possess little redox potential except for some specific clays with special functional groups, restricting their application in the removal and detoxification of oxidized metal ions, such as Cr(VI). In the present study, natural bentonite was modified with thiol and iron functional groups to improve both its adsorption performance and reduction ability. Material characterization techniques confirmed the success of grafting the thiol group and pillaring the reduced Fe in the clay. Batch adsorption tests demonstrated an enhanced Cr-adsorption capability of the functionalized bentonite. Moreover, the reduced product, Cr(III), was accumulated in the reaction system, indicating a decrease in Cr(VI) toxicity due to its reduction to Cr(III) by the reduced iron functional group in the modified clay. Pot experiments, using mustard as the target seedling, further demonstrated that the thiol/Fe-modified clay could efficiently stabilize and detoxify Cr(VI) in the soil, therefore inhibiting its accumulation in the crop tissues and ultimately improving plant growth. The results suggested that this novel clay modified with sulfhydryl and iron might be a promising material for the stabilization and toxicity alleviation of Cr(VI)-contaminated soils.

© 2016 Elsevier B.V. All rights reserved.

1. Introduction

Clay minerals have historically been used as adsorbents for heavy metal in removal processes because of their advantageous characteristics, including high specific surface area, ion exchange properties, low cost, natural abundance and environmental stability (Celis et al., 2000; Sarkar et al., 2013; Vasilyeva et al., 2014). Since natural clay usually suffers from poor binding strength and low metal selectivity, it typically requires functional modifications (Celis et al., 2000; Miličević et al., 2013; Sarkar et al., 2013). For example, the introduction of the thiol group (–SH) into clay minerals was explored to improve their specific binding capability toward various highly toxic heavy metal ions, such as Hg²⁺, Pb²⁺, and Cd²⁺ (Lagadic et al., 2001; Lee and Tiwari, 2012). Moreover, the incorporation of functional metal groups, such as iron (Fe(II)), might contribute to adding new characteristics to clay, enhancing sorptive capacity (Dousova et al., 2014) and reducing reactivity (Bhattacharyya and Sen Gupta, 2008; Bishop et al., 2014). These modifications would be highly beneficial for the remediation of soils contaminated by oxidative pollutants, such as hexavalent chromium (Cr(VI)).

Chromium (Cr) is one of the most common pollutants in groundwater, soil and sediments, and it presents one of the leading threats to human health (Dhal et al., 2013; Sarkar et al., 2013; Albanese et al., 2007). Trivalent chromium, Cr(III), and hexavalent chromium, Cr(VI), are two of the most stable and common forms of Cr in the environment. Compared to Cr(III), Cr(VI) is a more toxic and carcinogenic form, with a high mobility due to higher water solubility over the full pH range. In contrast, Cr(III) tends to readily precipitates and adsorbs on the soil surface (Barrera-Diaz et al., 2012; Dhal et al., 2013). The environmental contamination of Cr primarily arises from solid or liquid wastes from industrial activities, such as mining, electroplating, leather tanning, metal processing, as well as pigment and dye production (Albanese et al., 2013; Dhal et al., 2013). Soils in agricultural land close to industrial areas would be affected first and would result in severe heavy metals contamination (Krishna and Govil, 2004; Shao et al., 2014). As a non-essential element, Cr is significantly toxic to microorganisms and plants. This toxicity inhibits germination, obstructs root and seedling growth and development, induces leaf chlorosis and necrosis, and alters physiological and biochemical processes of plants (Singh et al., 2013).

Various techniques have been employed to detoxify and remove Cr from soils and ground water, including precipitation, reduction, extraction, and adsorption through chemical, electrochemical, or biological means (Barrera-Diaz et al., 2012; Dhal et al., 2013). Among these

* Corresponding author at: Institute of Geochemistry, Chinese Academy of Sciences, Guiyang 550081, China.

E-mail address: liuchengshuai@vip.gyig.ac.cn (C. Liu).

treatments, adsorption is a widely applied method due to its low cost and simplicity (Bhattacharyya and Gupta, 2006; Ghorbel-Abid et al., 2009). Additionally, natural clay minerals, with modified functional groups, have shown great potential in this application (e.g. Gladysz-Plaska et al., 2012; Li et al., 2016; Sarkar et al., 2013). Examples of these functional groups include sulfur and iron compounds, which have been shown to be active in the reduction of toxic Cr(VI) to the less toxic Cr(III) form (Barrera-Diaz et al., 2012). The combined modification of clay with thiol and iron groups, therefore, might result in an efficient alleviation of the Cr(VI) contamination problem. However, only few reports on the single modification of clay with a thiol or iron group to improve the adsorption performance have been published (Lee and Tiwari, 2012; Sarkar et al., 2013). Studies focusing on the combined modification or on the functionalization of clay for Cr(VI) reduction in contaminated soils have not been reported.

In this study, therefore, a novel functional clay modified with a thiol group and iron was developed and was used for the remediation of Cr(VI)-contaminated soils for mustard growth through the following processes: (i) the reduction and subsequent detoxification of Cr(VI) to Cr(III), and (ii) the adsorption of Cr(VI) and Cr(III) to stabilize the soils. The results are of great importance for safe agricultural production from Cr(VI)-contaminated soils.

2. Materials and methods

2.1. Materials

The clay used in the present study was Na-bentonite and was collected from Lin'an, Zhejiang, China. All of the chemicals for the clay modification and adsorption tests, including potassium dichromate ($K_2Cr_2O_7$), hydrochloric acid (HCl), cysteamine hydrochloride, sodium carbonate anhydrous (Na_2CO_3), ferrous sulfate ($FeSO_4$), etc., were of analytical grade and were obtained from Sigma-Aldrich Company, unless otherwise stated. A fresh stock solution of Cr(VI) was prepared by dissolving 1.47 g $K_2Cr_2O_7$ into 1 L deionized water (DI water) to yield an accurate concentration of 10 mmol/L Cr. The pH was adjusted to approximately 5.0 by HCl. As a control test, non-polluted soil was collected from a vegetable base site in the Baiyun District, Guangzhou, China. No Cr was detected in the soil by atomic absorption spectrometry after thoroughly digested in the HCl-HNO₃-HF-HClO₄ system. This soil was artificially contaminated in the laboratory by spiking with the prepared Cr(VI) solution to obtain a contamination level of 300 mg/kg.

2.2. Clay functionalization

The clay was first functionalized with mercaptamine to obtain thiol-modified clay using the following procedure. Na-bentonite was mixed with 20% HCl in a ratio of 1:10 (w:v) and then stirred at 80 °C for 4 h before being filtered by quantitative filter paper, dried and ground. Next, the bentonite powder was dispersed in DI water to yield a 2% suspension, which was then mixed with 10 g/L cysteamine hydrochloride in a ratio of 10:1 (v:v) and stirred for 4 h. After centrifugation at 8000 rpm for 10 min and washing with DI water several times, the modified clay was dried at 60 °C and ground to pass through a 200-mesh sieve.

To prepare the combined thiol/Fe-modified clay, a poly-hydroxyl iron solution was firstly prepared by the addition of 16 g Na_2CO_3 to 1.0 L of 0.1 mol/L $FeSO_4$. The mixture was stirred for 2 h and aged for 24 h at 60 °C. The freshly prepared poly-hydroxyl iron solution was then added to a 2.0% thiol-modified clay suspension at a ratio of 2:1 (v:v). The mixture was then stirred for 2 h and aged for 24 h at 60 °C. After centrifugation at 8000 rpm for 10 min, the sample was washed several times with DI water. After drying at 60 °C, the sample was ground to pass through a 200-mesh sieve, thus obtaining the combined thiol/Fe-modified clay.

2.3. Characterization of clays

In order to characterize the clay samples, the natural and modified clays were homogeneously ground into powder. The powder samples were characterized by scanning electron microscopy/energy dispersive spectroscopy (SEM-EDS), Fourier Transform infrared spectroscopy (FTIR) and X-ray photoelectron spectroscopy (XPS). The morphology and chemical compositions of the clay were investigated using SEM-EDS (Leica Stereo scan 400i series, USA), operating at 20 kV. For FTIR characterization, the powder samples were mixed with KBr powder and pressed into pellets. Each FTIR measurement (Bruker Vector 33, Germany) was recorded with 64 scans at a resolution of 4 cm^{-1} over a range of 4000 to 400 cm^{-1} . XPS analyses were performed on a Kratos Axis spectrometer (SHIMADZU, Japan) with Mg $K\alpha$ radiation ($h\nu = 1250.6$ eV). The pressure in the analysis chamber was $<1 \times 10^{-9}$ mbar, and the binding energies of the samples were calibrated by taking the carbon 1 s peak as a reference (284.8 eV).

2.4. Cr(VI) adsorption and reduction experiments

The adsorptive capacities of the modified clays for Cr(VI) were investigated through batch experiments. During the adsorption experiments, clays were added to the prepared working solutions at initial Cr(VI) concentrations of 2.0 mmol/L, thereby obtaining clay dosages of 3.0 g/L. Then, the pH of the suspension was adjusted with 0.5 M NaOH or HCl to reach the target pH values. After allowing adsorption equilibration at 150 rpm shaking for 24 h or at regular time intervals, the clays were separated from the solution via centrifugation at 4500 rpm for 5 min, and the supernatant was filtered through 0.45- μm hydrophilic PTFE filters (Millipore, MA, USA) to determine the Cr concentrations. All of the experiments were conducted in triplicate, and the results are provided with standard deviation values.

2.5. Mustard seed germination experiments

To investigate the primary ecotoxicological effect of Cr stress on mustard seed germination (Dell'Aquila, 2009; Bae et al., 2014), seeds of *Brassica juncea* in uniform size were placed in petri dishes lined with three layers of qualitative filter paper (Wet Strengthened Grades, Whatman, UK). The filter paper layers were previously moistened with 15 mL of a Cr(VI) solution at concentrations of 0, 90, 300, and 600 mg/L. Triplicate experiments were conducted for each treatment. All of the petri dishes were incubated in a climate incubator (MGC-300H, Shanghai, China) at 25 °C for 7 days, with a 16/8-h photoperiod and a humidity of 75%. The germination ratio, root length, and root dry weight were measured at the end of the experiments.

2.6. Experimental remediation of Cr(VI)-contaminated soil with clays for mustard growth

Pot experiments for mustard growth were performed to evaluate the ability of the clays to remediate Cr(VI)-contaminated soil. The thiol/Fe-modified clay was dosed and homogeneously mixed into the Cr-contaminated soil at treatment levels of 0, 1.0, 5.0, 10, 20 g/kg. The treated soils were then mixed with DI water in a ratio of 1:2.5 (w:v) and equilibrated for 30 days before use.

When the treated soils were ready and no water logging was found on the surface, selected plump mustard seeds of uniform size which looked healthy and vigorous were sown. Twelve seeds were placed in each pot, and the seeds were covered with the homogeneous soils up to a thickness of 1–2 times the seed size (~5 mm). The pots were covered with plastic film to preserve the humidity for seed germination until the second euphylla started to expand. After the fourth euphylla developed, the seedlings were removed, and only 6 strains demonstrating positive and similar growth conditions were retained in each pot. Soils were watered every day to maintain the water content at

approximately 60%–70% of the effective water holding capacity. During the entire growth period, pests were artificially avoided, and the growth was observed and recorded daily.

The crops were harvested after incubation for 40 days. Plants were firstly washed with tap water to remove the soils and any adhered Cr on the plant surface, followed by triple washes with DI water, which was drained. Subsequently, the plant height, stem diameter and leaf area (length and width) were measured. The roots and shoots were baked at 50 °C until reaching a constant weight to determine fresh and dry weights. The dried roots and shoots were then ground, digested with concentrated nitric acid and perchloric acid (3:1, v:v), and measured for total Cr concentration.

2.7. Cr determination and data analysis

The total Cr concentration in solution was determined using a graphite atomic absorption spectrometer (Jena, Zeenit 60, Germany). Cr(VI) was determined using the colorimetric method by reacting with diphenylcarbazide in acid solution, according to standard methods (Clescerl et al., 1999). The data reported in this study are the mean values with the standard deviation based on the triplicate results. Reagent blanks and analytical duplicates were also used where appropriate to ensure accuracy and precision in the analysis. Microsoft Excel 2010, SPSS 13.0 and Origin 8 were employed for data analysis and graph plotting.

3. Results and discussion

3.1. Changes in structure and functional groups of clay after modification

The FTIR spectra over the range of 300–4000 cm^{-1} of unmodified bentonite, thiol-bentonite, and thiol/Fe-bentonite are recorded (Fig. 1).

The absorption bands at 2564 cm^{-1} (Fig. 1B) and 2554 cm^{-1} (Fig. 1C) can be identified in the spectra of thiol-modified clay and thiol/Fe-modified clay, which could be assigned to the stretching vibrations of sulfhydryl groups, thus providing evidence of the grafted thiol-group (Guimaraes et al., 2009; Hua and Li, 2014; Wu et al., 2012). Compared with thiol-bentonite, a new absorption band at approximately 1493 cm^{-1} (Fig. 1C), which is typically attributed to the Fe–O stretching vibration (Seehra et al., 2004), is observed in the spectrum of thiol/Fe-bentonite, indicating the successful Fe pillaring into the modified clay.

Fig. 2 shows the typical SEM images of the raw and modified clay materials. The image of the raw clay shows a rigid shape (Fig. 2A). The images for the thiol-modified and thiol/Fe-modified clays (Figs. 2B and C) show that the surfaces appear to be markedly coarser and more porous with notable fragments. These observations are similar to those found by others during modification of clay minerals (Gan et al., 2012; Wang and Wang, 2008), indicating the introduction of functional groups onto the clay material.

Changes in the elemental composition of the clays from the EDS study further indicate the chemical modification (Table 1). After the acid wash and thiol-group introduction, the data indicated that the thiol-modified clay contained fewer cations, such as Na, K, and Ca. Due to inherent errors, including the possible anomalies generated by surface heterogeneity and poor detection, the elemental composition accuracy was below 5.0% (Oztop and Shahwan, 2006). For the thiol/Fe-modified clay, a significant increase (approximately 15%) of Fe content was found, providing evidence for Fe incorporation. Accordingly, the ratio of most of the other elements decreased for the thiol/Fe-modified clay. XPS spectra further confirmed the presence of S and Fe, along with the reduced Fe^{2+} in the thiol/Fe-modified clay. The presence of the elements Fe, S, C, and O are shown in the spectrum (Fig. 3A). Fig. 3B shows the high-resolution XPS survey spectrum of the Fe peaks. The observed binding energy of Fe 2p_{1/2} at 725.0 and 712.7 eV indicates the presence

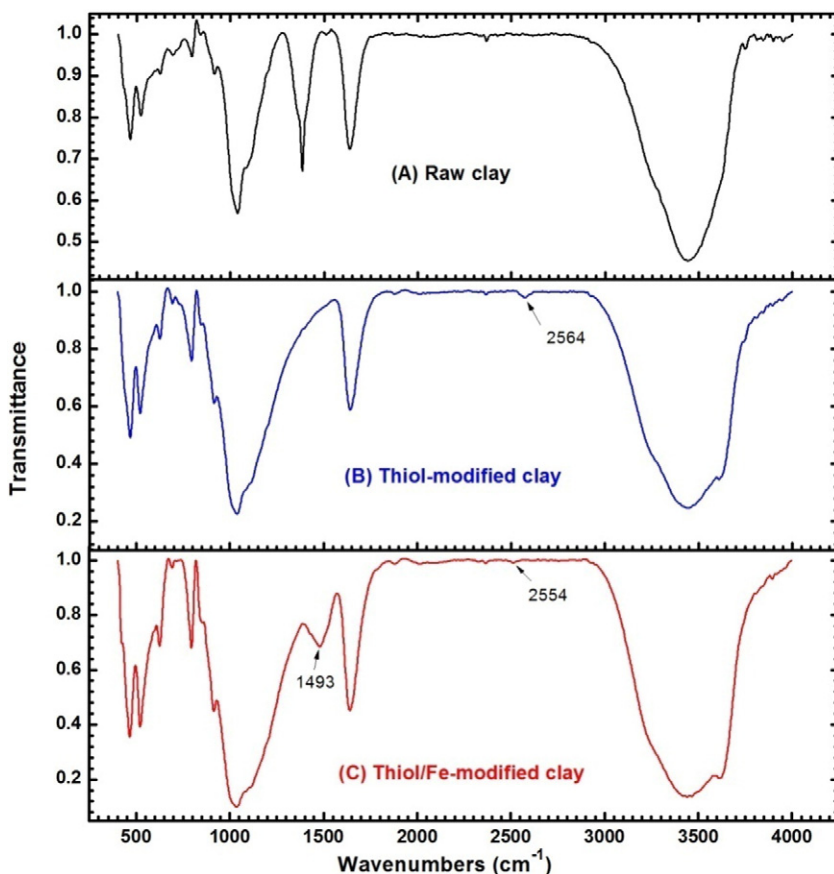


Fig. 1. FTIR spectra of raw clay (A), thiol-modified clay (B), and thiol/Fe-modified clay (C).

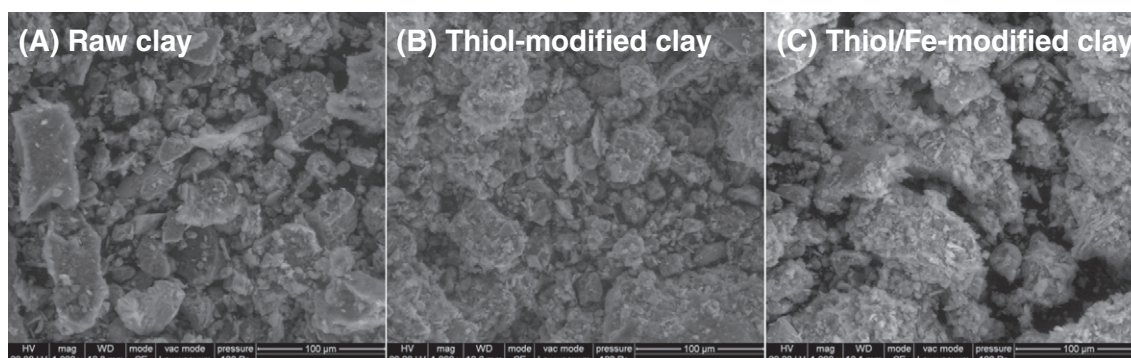


Fig. 2. SEM images of clay and modified clay materials.

of reduced Fe^{2+} , and the binding energy of $\text{Fe } 2p_{1/2}$ at 728.0 and 714.7 eV indicates the presence of Fe^{3+} .

3.2. Adsorption and reduction of Cr(VI) by modified clays

Batch experiments were conducted in solution to investigate the adsorption behaviors of the raw and modified clays, and the results are shown in Fig. 4A. Overall, thiol resulted in an obvious increase in the Cr removal rates compared with the clay mineral, and the thiol/Fe-modification further increased the rate. Under the acidic conditions at pH 3.0, 3.0 g/L of raw clay dosage could remove approximately 60% of the initial Cr from the solution, which was resulted from the intrinsic adsorption properties of the bentonite (Hamidpour et al., 2010). Due to the thiol-modification, the removal rate was increased to approximately 70%. The combined modified clay further improved the removal rate by nearly an additional 20% over that of thiol-modified clay.

As displayed in Fig. 4B, the removal rate of Cr(VI) with 3.0 g/L thiol/Fe-modified clay occurred quickly, especially in the first 40 min. The removal rate resulted from adsorption with the modified clay and reduction by the Fe(II) and sulfhydryl groups. Fig. 4B also shows that Cr(III) was produced as a result of the reduction of Cr(VI) during the experimental process. One interesting phenomena observed from Fig. 4B is that the production rate of Cr(III) was slower in the first 40 min in which the Cr(VI) concentrations sharply decreased. In the latter 80 min, however, the production rate of Cr(III) increased significantly, while the removal rate of Cr(VI) in the solution slowly decreased. These results suggest that the adsorption of Cr(VI) by thiol/Fe-modified clay initially occurred efficiently but was later inhibited due to saturation of the adsorption process. The reduction rate of Cr(VI) was initially slow because of insufficient contact of soluble Cr(VI) with the reduction groups in the modified clay. At the later reaction stage, the adsorbed Cr(VI) was gradually reduced on or in the modified clay by the reducing groups and then released into the solution during the equilibrium of the adsorption/desorption of Cr to clay. The reduction of Cr(VI) to Cr(III) by the thiol/Fe-modified clay was also confirmed by XPS analysis (Fig. S1 in Supplementary Information (SI)). The XPS spectrum shows the binding energy of $\text{Cr}^{3+} 2p_{1/2}$ as 587.1 eV, which indicated the presence of Cr in

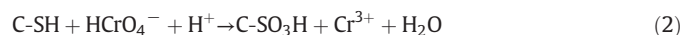
the thiol/Fe-modified clay and, more importantly, confirmed the reduction of Cr(VI) to Cr(III) by the thiol/Fe-modified clay.

Notably, a decrease in the Cr removal rate was observed in all three clays when the initial pH of the solution increased (Fig. 4A). This result is consistent with other published reports in which Cr(VI) adsorption decreased with the increase in pH (Bayrak et al., 2006; Sarkar et al., 2010; Weng et al., 2008). Because of the ionization of the functional groups at the edge of the clay, the surface charge of the clays is typically pH-dependent. The entire pH range was tested in the present study, and the dominant Cr(VI) species was HCrO_4^- or CrO_4^{2-} whose sorption was primarily through salt formation (Sarkar et al., 2010), which is explained by equation (1) (Kimbrough et al., 1999):



where C represents the clay. Thus, the more positively charged clays over the low pH range are preferred by the Cr(VI) anion through electrostatic interactions (Bayrak et al., 2006; Weng et al., 2008).

Thiol-modified clays enhanced the removal rates by approximately 10% over the entire tested pH range, except for a slight reduction at pH 9.0 (Fig. 3A). These increases could not be attributed to ion exchange with the grafted -SH group because the dominant Cr(VI) species in the solution were negatively charged anions. The -SH group however was shown to be active in reducing Cr(VI) anions to Cr(III) ions, as illustrated in equation (2) (Shevchenko et al., 2008; Szulczewski et al., 2001). The produced Cr^{3+} would then be immobilized primarily through ion exchange, which would increase the total Cr removal rates in the solution, as shown in the reactions below (Hua and Li, 2014):



The thiol/Fe-modified clay (due to a further modification with the Fe group) increased this value by 3–5% over the pH range of 4.5–9.0. In contrast, the enhancement was dramatically higher at the lowest pH of 3.0 (Fig. 4A). Unlike the thiol group, the Fe could directly improve the Cr removal rate through anion exchange, as represented by equation (4) (Ajouyed et al., 2010). Moreover, the ferrous group was pillared into the clay as an efficient reducing agent. In the combined modified clay, therefore, both the thiol and the incorporated Fe(II) groups led to the reduction of Cr(VI) to Cr(III), and these reactions are presented in equations (5) and (6) (Kiser and Manning, 2010). Further, higher removal rates of total Cr were obtained with the thiol/Fe-modified clays compared with unmodified clays.

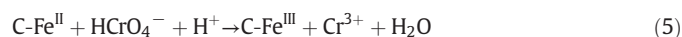
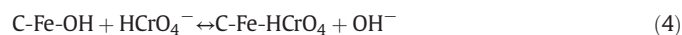


Table 1
Element percentages in the clay materials (%).

Element	Raw clay	Thiol-modified clay	Fe/thiol-modified clay
O	67.94	71.88	65.74
Na	1.46	0.63	1.93
Mg	1.43	1.48	0.97
Al	5.61	6.07	4.07
Si	20.94	17.98	11.06
K	0.65	0.41	0.34
Ca	1.25	0.63	0.46
Fe	0.72	0.92	15.43

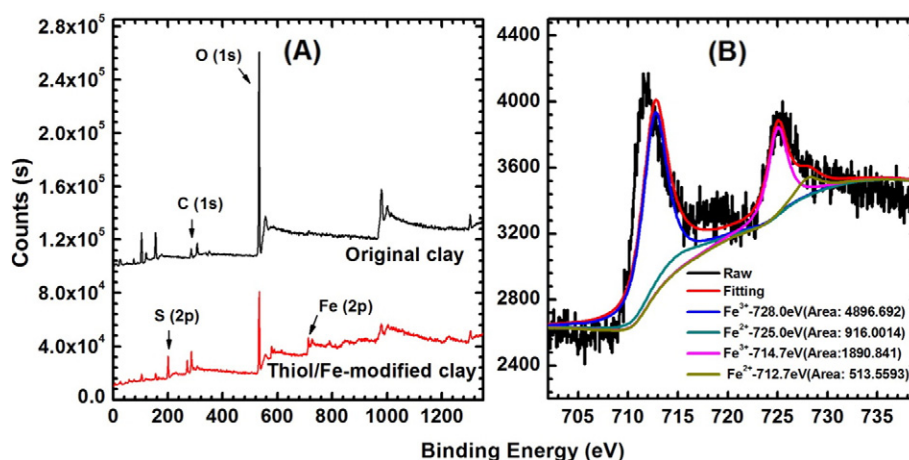


Fig. 3. XPS spectrum of the thiol/Fe-modified clay (A) and high-resolution XPS spectrum of Fe of the thiol/Fe-modified clay.



3.3. Mustard growth on the modified clay-amended Cr-contaminated soil

The mustard *Brassica juncea* is known to potentially accumulate heavy metals (Clemente et al., 2005) and was employed in this study as an indicator for Cr stress in the Cr(VI)-contaminated soil for mustard growth. According to the germination test results, as summarized in Table S1 in the SI, Cr(VI) contamination greatly inhibited seed germination and root development. As an important developmental stage in the plant life cycle, the seed is highly protected against and sensitive to environmental stresses (Li et al., 2005). The germination rate of the selected seeds decreased with increasing Cr(VI) contamination. In comparison, the root length was most sensitive to Cr(VI) stress, as demonstrated by the dramatic shortening from 32 mm to <1.0 mm under 90 mg/L of Cr(VI) pollution. Accordingly, the dry weight of the developed root also decreased significantly.

The effect of Cr(VI)-contaminated soil remediation with thiol/Fe-modified clay on plant growth was then evaluated by using mustard as the indicative crop. The results suggest enhanced growth of the mustard and inhibited Cr accumulation in the crop tissues (Figs. 5 and 6 for the biomass and Cr accumulation in mustard seedlings, respectively). Enhanced mustard growth could be observed from the harvested

plant photos in the thiol/Fe-modified clay treated soil treated, compared with the untreated contaminated soil (Fig. S2 in SI), as the mustard cultivated in the Cr(VI)-contaminated soil was the shortest with the least biomass, while, due to the treatment of amended thiol/Fe-modified clay, the harvested mustard developed significantly more branches and had a higher biomass. A greater number (and quality) of plants were obtained at the 20 g/kg dose of the amended thiol/Fe-modified clay than at the lower dosage of 5.0 g/kg.

The fresh weight (Fig. 5A) and dry weight (Fig. 5B) of the harvested plants after 40 days of cultivation provided additional information on the efficiency of the soil Cr detoxicated by thiol/Fe-modified clay. Briefly, the artificially Cr-contaminated soil demonstrated its threat to crop growth because the fresh and dry weight of the untreated contaminated group (0 g/kg modified clay) was approximately 46% and 18% of the clean soil (CK), respectively. The modified clay performed well in treating the Cr contamination, as indicated by the data in which a dosage of only 1.0 g/kg to the contaminated soil improved the crop growth by 22% or 133%, as estimated by fresh or dry weight, respectively. When the dosage of modified clay was increased to 20 g/kg, the harvested biomass from the treated soil recovered to nearly the same level as that from the clean soil.

The Cr concentrations in the mustard shoots and roots were further studied to more directly evaluate the Cr accumulation in the mustard from the contaminated soils treated with different dosages of thiol/Fe-

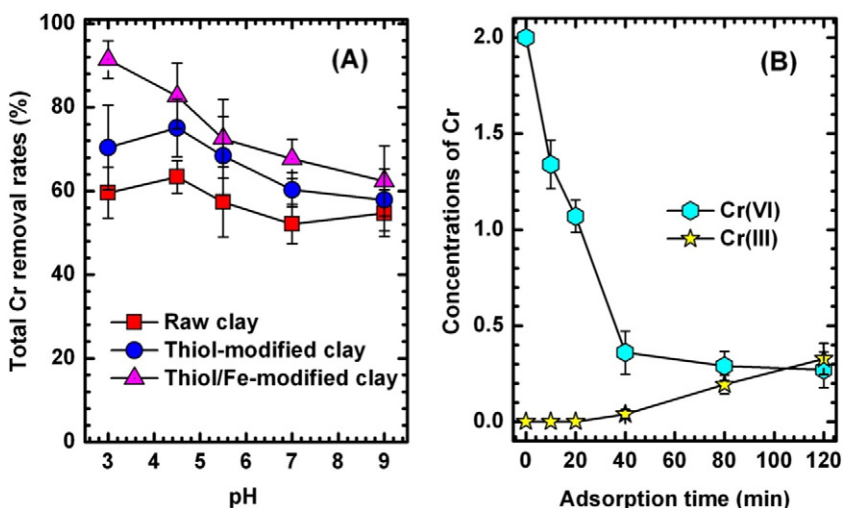


Fig. 4. The adsorptive removal rates of Cr (2 mmol/L) by clay and modified clay materials (3.0 g/L) at different pH values (A), and the changes in the concentration of the residual Cr(VI) and produced Cr(III) during the adsorption process of 2 mmol/L Cr with 3.0 g/L thiol/Fe-modified clay (B).

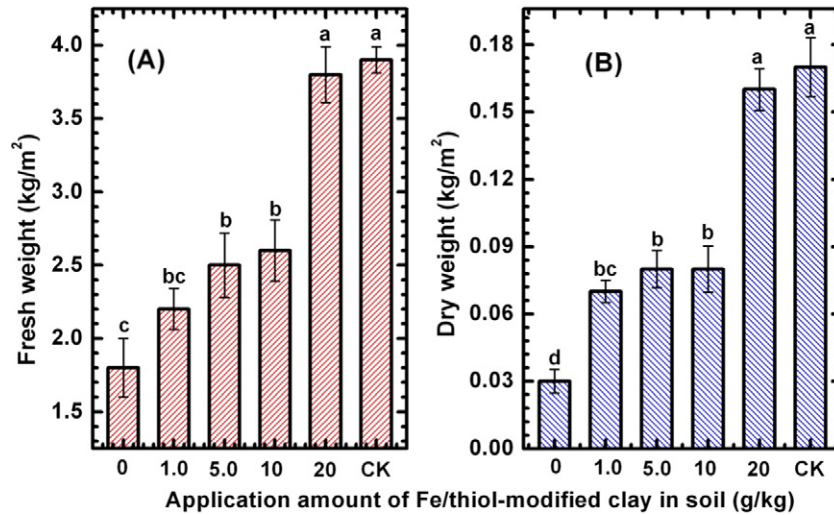


Fig. 5. The fresh weight (A) and dry weight (B) of mustard after growth for 40 days in pot experiments with the Cr-contaminated soils (300 mg/kg) treated with different contents of thiol/Fe-modified clay. CK represents the mustard experiments with the clean soils. The average value within the same treatment with different lowercase letters (i.e., a or b) is significantly different at $p < 0.05$ and $p < 0.01$, whereas the average value within the same treatment with identical lowercase letters is not significantly different at $p = 0.05$.

modified clay (Fig. 6). When the contaminated soil was treated with 1.0 g/kg of the modified clay, the Cr contents in the crop shoots and leaves were reduced by 27% compared to that in the contaminated soil without modified clay treatment. At higher dosages of modified clay, the Cr contents in the mustard shoots and leaves were further reduced. When treated with 20 g/kg of modified clay, the Cr content decreased by >55%. The roots might have been less sensitive to the remediation effect by the modified clay, but the Cr-accumulated contents in the roots still decreased as the dosage of the modified clays increased. At the dosage of 20 g/kg, a 33% reduction of Cr accumulation was achieved compared with the contaminated soil without treated modified clay.

3.4. Cr toxicity alleviation mechanisms in the soils by the modified clay

Clay minerals have been considered as one of the physical barriers for heavy metal immobilization in soils (Mulligan et al., 2001). For example, in addition to bentonite, artificially synthesized zeolitic material has also been shown to be effective in restraining the leaching of multiple types of heavy metals from polluted soils (Querol et al., 2006).

Vermiculite was also applied as a possible treatment for contaminated soils to reduce the uptake of metal pollutants by plants (Malandrino et al., 2011). In the present study, however, the thiol/Fe-modified bentonite served as a physical adsorbent and could reduce Cr(VI) to Cr(III), which is relatively less mobile in the environment and less toxic to plants and other living organisms.

With the addition of this modified clay into the Cr-contaminated soil, therefore, it was found that crop growth recovered, and the uptake of Cr by the plant was significantly reduced. The obtained thiol/Fe-modified clay would be an important material for the stabilization and detoxification of heavy metals and oxidative pollutants. The results obtained in this study provide a potentially promising solution for supporting agricultural production in Cr-contaminated soils.

4. Conclusions

A novel clay modified with sulfhydryl and iron for the adsorption and reduction of Cr(VI) was successfully prepared in this study. FTIR and SEM-EDS characterization of the thiol/Fe-modified clay confirmed

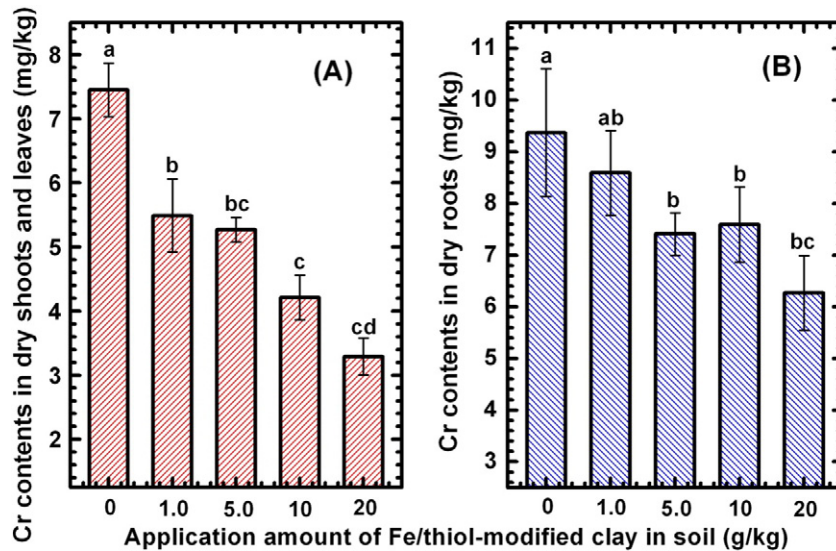


Fig. 6. The accumulated Cr contents in the dry shoots and leaves (A) and dry roots (B) after growth for 40 days in pot experiments with the soils treated with different concentrations of thiol/Fe-modified clay applications. The average value within the same treatment with different lowercase letters (i.e., a or b) is significantly different at $p < 0.05$ and $p < 0.01$, whereas the average value within the same treatment with identical lowercase letters is not significantly different at $p = 0.05$.

the grafting of the thiol group and the incorporation of the Fe in the modified clay. The thiol/Fe-modified clay showed an enhanced performance for the adsorptive removal of Cr(VI) from the aqueous solution compared with the raw bentonite. Further, the reductive groups of sulfhydryl and Fe(II) in the modified clay led to the reduction of Cr(VI) to Cr(III), which further alleviated the toxicity of Cr. The obtained thiol/Fe-modified clay can remediate the Cr-contaminated soil by stabilizing and alleviating the toxicity of the Cr ions. In the Cr-contaminated soil, the growth of mustard seeds was significantly hindered, and high concentrations of Cr accumulated in the roots and leaves of the plant. When the Cr-contaminated soil was treated with the thiol/Fe-modified clay, the biomass of the planted mustard increased, and the accumulated Cr content in the organs of the mustard seedlings decreased. As the dosage of the treated thiol/Fe-modified clay increased, the mustard growth in the Cr-contaminated soil recovered to levels similar to that when planted in clean soil. The uptake of Cr by the plant was also significantly reduced.

Acknowledgments

We acknowledge the financial supports provided by the National Natural Science Foundation of China (41673135), the Guangdong Natural Science Foundation of China (S2013050014266), the Science and Technology Planning Project of Guangdong Province (2016B020242006 and 2014A020216001), the Scientific Platform and Innovation Capability Construction Program of GDAS (2016GDASPT-0212), and the One Hundred Talents Program of The Chinese Academy of Sciences.

Appendix A. Supplementary data

Supplementary data to this article can be found online at doi:10.1016/j.gexplo.2016.10.014.

References

- Ajouyed, O., Hurel, C., Ammari, M., Ben Allal, L., Marmier, N., 2010. Sorption of Cr(VI) onto natural iron and aluminum (oxy)hydroxides: Effects of pH, ionic strength and initial concentration. *J. Hazard. Mater.* 174, 616–622.
- Albanese, S., De Vivo, B., Lima, A., Cicchella, D., 2007. Geochemical background and baseline values of toxic elements in stream sediments of Campania region (Italy). *J. Geochem. Explor.* 93, 21–34.
- Albanese, S., Iavazzo, P., Adamo, P., Lima, A., De Vivo, B., 2013. Assessment of the environmental conditions of the Sarno river basin (south Italy): a stream sediment approach. *Environ. Geochem. Health* 35, 283–297.
- Bae, J., Mercier, G., Watson, A.K., Benoit, D.L., 2014. Seed germination test for heavy metal phytotoxicity assessment. *Can. J. Plant Sci.* 94, 1519–1521.
- Barrera-Diaz, C.E., Lugo-Lugo, V., Bilyeu, B., 2012. A review of chemical, electrochemical and biological methods for aqueous Cr(VI) reduction. *J. Hazard. Mater.* 223, 1–12.
- Bayrak, Y., Yesiloglu, Y., Gecgel, U., 2006. Adsorption behavior of Cr(VI) on activated hazelnut shell ash and activated bentonite. *Microporous Mesoporous Mater.* 91, 107–110.
- Bhattacharyya, K.G., Gupta, S.S., 2006. Adsorption of chromium (VI) from water by clays. *Ind. Eng. Chem. Res.* 45, 7232–7240.
- Bhattacharyya, K.G., Sen Gupta, S., 2008. Adsorption of a few heavy metals on natural and modified kaolinite and montmorillonite: a review. *Adv. Colloid Interf. Sci.* 140, 114–131.
- Bishop, M.E., Glasser, P., Dong, H.L., Arey, B., Kovarik, L., 2014. Reduction and immobilization of hexavalent chromium by microbially reduced Fe-bearing clay minerals. *Geochim. Cosmochim. Acta* 133, 186–203.
- Celis, R., Hermosin, M.C., Cornejo, J., 2000. Heavy metal adsorption by functionalized clays. *Environ. Sci. Technol.* 34, 4593–4599.
- Clemente, R., Walker, D.J., Bernal, M.P., 2005. Uptake of heavy metals and As by *Brassica juncea* grown in a contaminated soil in Aznalcollar (Spain): The effect of soil amendments. *Environ. Pollut.* 138, 46–58.
- Clescerl, L.S., Greenberg, A.E., Eaton, A.D., 1999. Standard Methods for the Examination of Water and Wastewater. American Public Health Association.
- Dell'Aquila, A., 2009. Digital imaging information technology applied to seed germination testing. A review. *Agronomy of Sustainable Development* 29, 213–221.
- Dhal, B., Thatoi, H.N., Das, N.N., Pandey, B.D., 2013. Chemical and microbial remediation of hexavalent chromium from contaminated soil and mining/metallurgical solid waste: A review. *J. Hazard. Mater.* 250, 272–291.
- Dousova, B., Fuitova, L., Kolousek, D., Lhotka, M., Grygar, T.M., Spurna, P., 2014. Stability of iron in clays under different leaching conditions. *Clay Clay Miner.* 62, 145–152.
- Gan, H.H., Zhang, G., Zhang, Y.L., Guo, Y.D., 2012. Adsorption of Rhodamine B from aqueous solution onto sepiolite modified by cetyltrimethylammonium bromide. *Desalin. Water Treat.* 45, 112–119.
- Ghorbel-Abid, I., Jrad, A., Nahdi, K., Trabelsi-Ayadi, M., 2009. Sorption of chromium (III) from aqueous solution using bentonitic clay. *Desalination* 246, 595–604.
- Gladysz-Plaska, A., Majdan, M., Pikus, S., Sternik, D., 2012. Simultaneous adsorption of chromium (VI) and phenol on natural red clay modified by HDTMA. *Chem. Eng. J.* 179, 140–150.
- Guimaraes, A.D.F., Ciminelli, V.S.T., Vasconcelos, W.L., 2009. Smectite organofunctionalized with thiol groups for adsorption of heavy metal ions. *Appl. Clay Sci.* 42, 410–414.
- Hamidpour, M., Afyuni, M., Kalbasi, M., Khoshgoftarmans, A.H., Inglezakis, V.J., 2010. Mobility and plant-availability of Cd(II) and Pb(II) adsorbed on zeolite and bentonite. *Appl. Clay Sci.* 48, 342–348.
- Hua, R., Li, Z.K., 2014. Sulfhydryl functionalized hydrogel with magnetism: synthesis, characterization, and adsorption behavior study for heavy metal removal. *Chem. Eng. J.* 249, 189–200.
- Kimbrough, D.E., Cohen, Y., Winer, A.M., Creelman, L., Mabuni, C., 1999. A critical assessment of chromium in the environment. *Crit. Rev. Environ. Sci. Technol.* 29, 1–46.
- Kiser, J.R., Manning, B.A., 2010. Reduction and immobilization of chromium(VI) by iron(II)-treated faujasite. *J. Hazard. Mater.* 174, 167–174.
- Krishna, A.K., Govil, P.K., 2004. Heavy metal contamination of soil around Pali industrial area, Rajasthan, India. *Environ. Geol.* 47, 38–44.
- Lagadic, I.L., Mitchell, M.K., Payne, B.D., 2001. Highly effective adsorption of heavy metal ions by a thiol-functionalized magnesium phyllosilicate clay. *Environ. Sci. Technol.* 35, 984–990.
- Lee, S.M., Tiwari, D., 2012. Organo and inorgano-organo-modified clays in the remediation of aqueous solutions: An overview. *Appl. Clay Sci.* 59–60, 84–102.
- Li, W.Q., Khan, M.A., Yamaguchi, S., Kamiya, Y., 2005. Effects of heavy metals on seed germination and early seedling growth of *Arabidopsis thaliana*. *Plant Growth Regul.* 46, 45–50.
- Li, W.B., Meng, Z.F., Liu, Z., Chen, H.Y., Wu, Q., Xu, S.E., 2016. Chromium (VI) adsorption characteristics of bentonite under different modification patterns. *Pol. J. Environ. Stud.* 25, 1075–1083.
- Malandrino, M., Abollino, O., Buoso, S., Giacomino, A., La Gioia, C., Mentasti, E., 2011. Accumulation of heavy metals from contaminated soil to plants and evaluation of soil remediation by vermiculite. *Chemosphere* 82, 169–178.
- Miličević, S., Milošević, V., Povrenović, D., Stojanović, J., Martinović, S., Babić, B., 2013. Removal of heavy metals from aqueous solution using natural and Fe (III) oxyhydroxide clinoptilolite. *Clay Clay Miner.* 61, 508–516.
- Mulligan, C.N., Yong, R.N., Gibbs, B.F., 2001. Remediation technologies for metal-contaminated soils and groundwater: an evaluation. *Eng. Geol.* 60, 193–207.
- Oztop, B., Shahwan, T., 2006. Modification of a montmorillonite-illite clay using alkaline hydrothermal treatment and its application for the removal of aqueous Cs⁺ ions. *J. Colloid Interface Sci.* 295, 303–309.
- Querol, X., Alastuey, A., Moreno, N., Alvarez-Ayuso, E., Garcia-Sanchez, A., Cama, J., Ayora, C., Simon, M., 2006. Immobilization of heavy metals in polluted soils by the addition of zeolitic material synthesized from coal fly ash. *Chemosphere* 62, 171–180.
- Sarkar, B., Xi, Y.F., Megharaj, M., Krishnamurti, G.S.R., Rajarathnam, D., Naidu, R., 2010. Remediation of hexavalent chromium through adsorption by bentonite based Arquad (R) 2HT-75 organoclays. *J. Hazard. Mater.* 183, 87–97.
- Sarkar, B., Naidu, R., Megharaj, M., 2013. Simultaneous adsorption of tri- and hexavalent chromium by organoclay mixtures. *Water Air Soil Pollut.* 224, 1704–1713.
- Seehra, M.S., Roy, P., Raman, A., Manivannan, A., 2004. Structural investigations of synthetic ferrihydrite nanoparticles doped with Si. *Solid State Commun.* 130, 597–601.
- Shao, X.X., Huang, B.A., Zhao, Y.C., Sun, W.X., Gu, Z.Q., Qian, W.F., 2014. Impacts of human activities and sampling strategies on soil heavy metal distribution in a rapidly developing region of China. *Ecotoxicol. Environ. Saf.* 104, 1–8.
- Shevchenko, N., Zaitsev, V., Walcarius, A., 2008. Bifunctionalized mesoporous silicas for Cr(VI) reduction and concomitant Cr(III) immobilization. *Environ. Sci. Technol.* 42, 6922–6928.
- Singh, H.P., Mahajan, P., Kaur, S., Batish, D.R., Kohli, R.K., 2013. Chromium toxicity and tolerance in plants. *Environ. Chem. Lett.* 11, 229–254.
- Szulgowski, M.D., Helmke, P.A., Bleam, W.F., 2001. XANES spectroscopy studies of Cr(VI) reduction by thiols in organosulfur compounds and humic substances. *Environ. Sci. Technol.* 35, 1134–1141.
- Vasilyeva, M.A., Gusev, Y.A., Shtyrlin, V.G., Gutina, A.G., Puzenko, A., Ishai, P.B., Feldman, Y., 2014. Dielectric relaxation of water in clay minerals. *Clay Clay Miner.* 62, 62–73.
- Wang, L., Wang, A.Q., 2008. Adsorption properties of Congo Red from aqueous solution onto surfactant-modified montmorillonite. *J. Hazard. Mater.* 160, 173–180.
- Weng, C.H., Sharma, Y.C., Chu, S.H., 2008. Adsorption of Cr(VI) from aqueous solutions by spent activated clay. *J. Hazard. Mater.* 155, 65–75.
- Wu, Z.M., Cheng, Z.H., Ma, W., 2012. Adsorption of Pb(II) from glucose solution on thiol-functionalized cellulosic biomass. *Bioresour. Technol.* 104, 807–809.