



## Remediation of copper polluted red soils with clay materials

Gangya Zhang<sup>1,\*</sup>, Yunqing Lin<sup>2</sup>, Mingkuang Wang<sup>3</sup>

1. State Key Laboratory of Soil and Sustainable Agriculture, Institute of Soil Science, Chinese Academy of Sciences, Nanjing 210008, China. E-mail: [linyuning886@163.com](mailto:linyuning886@163.com)

2. Graduate School of the Chinese Academy of Sciences, Beijing 100049, China

3. Department of Agricultural Chemistry, "National" Taiwan University, Taipei 10617, Taiwan

Received 01 April 2010; revised 13 June 2010; accepted 20 July 2010

### Abstract

Attapulgite and montmorillonite were utilized to remediate heavy metal polluted red soils in Guixi City, Jiangxi Province, China. The effects of clay minerals on availability, chemical distribution, and biotoxicity of Cu and Zn were evaluated. The results provided a reference for the rational application of clay materials to remediate heavy metal contaminated soils. From the sorption experiment, the maximum adsorbed Cu<sup>2+</sup> by attapulgite and montmorillonite was 1501 and 3741 mg/kg, respectively. After polluted red soil was amended with attapulgite or montmorillonite and cultured at 30 and 60 days, soil pH increased significantly compared to the control. An 8% increase in the amount of montmorillonite in soil and 30 days incubation decreased acid exchangeable Cu by 24.7% compared to the control red soil. Acid exchangeable Cu decreased with increasing amounts of attapulgite and montmorillonite, with best remediation effect reached at a dose of 8%. Results also showed that the Cu poisoning effect on earthworms was reduced with the addition of attapulgite and montmorillonite. Montmorillonite showed the best effect, with the addition of a 2% dose the mortality of earthworms decreased from 60% to zero compared to the control. Our results indicated that the bioavailability of Cu in soils was reduced more effectively with the application of montmorillonite than attapulgite.

**Key words:** amendments; montmorillonite; attapulgite; heavy metal polluted soil; remediation

**DOI:** 10.1016/S1001-0742(10)60431-7

**Citation:** Zhang G Y, Lin Y Q, Wang M K, 2011. Remediation of copper polluted red soils with clay materials. *Journal of Environmental Sciences*, 23(3): 461–467

### Introduction

Despite efforts by many countries to reduce heavy metal discharge, heavy metal disposal is still increasing worldwide. Although soil itself can reduce the mobility and bioavailability of heavy metals, immediate remediation measures in many heavily contaminated areas are still required. It is important, therefore, to develop an effective and economic *in situ* technology to immobilize copper pollutants (Cheng and Hseu, 2002). Immobilization of heavy metals is a promising alternative to the generally expensive and disruptive conventional remediation techniques for reduction in the environmental health risks of heavy metal contaminated sites (Geebelen et al., 2002; Vangronsveld and Cunningham, 1998). Many natural or synthetic materials such as phosphate rocks, zeolites (Querol et al., 2006), manganese, aluminium oxides and oxy-hydroxides, alkaline agents such as lime (Gray et al., 2006), clay minerals, and organic materials (i.e., compost, peat, and manure) (Liu et al., 2009) have been tested in the last decade to evaluate their ability to immobilize toxic trace metals. In general, these treatments lessen the risk of polluted soils

by limiting metal leaching and bioavailability. Nevertheless, some have been found to increase metal solubility, particularly those treatments based on organic materials (Narwal and Singh, 1998), manganese oxides (McBride and Martínez, 2000), and phosphates (Cao et al., 2009). It is important, therefore, to choose an environmentally friendly amendment that can remediate soils polluted with heavy metals while not producing secondary pollutants.

In spite of the large amount of research on the use of different materials as soil amendments, little research on clay minerals has been conducted. In the present study, clay minerals were selected as amendments as they are integral components of soil ecosystems and are widely available, non-toxic, and inexpensive (Pandian et al., 2001; Toribio and Romanyà, 2006). Due to the hydrophilicity of clays, their addition to soils can restore and enhance plant growth, especially when the clays have large cation exchange capacity (CEC) (Churchman, 2002). Most of the physicochemical properties of clays come from their laminar morphology (phyllosilicates) and their small grain size (< 2 μm), as well as the isomorphous substitutions originated from the electric charge of the layers. As a consequence, they can interact with heavy metals by adsorption, ion

\* Corresponding author. E-mail: [gyzhang@issas.ac.cn](mailto:gyzhang@issas.ac.cn)

jesc.ac.cn

exchange reactions, and the formation of inner-sphere complexes (Celis et al., 2000). Heavy metal adsorption by clay minerals is generally activated at permanent negative charge sites (Kim et al., 2005). There is, however, little information on the immobilization of heavy metals from soils with the addition of clay minerals. It is worth noting that not only dissolved heavy metals but also some nutrient cations will be absorbed by some clay minerals. Further studies on the remediation mechanisms and effectiveness of clay minerals need to be done.

In this study, attapulgite and montmorillonite were the chosen amendments. A comprehensive evaluation of the effectiveness of these two clay minerals was made by means of an adsorption experiment, indoor incubation experiment, and biotoxicity experiment. The results obtained provide a theoretical basis for the rational application of clay materials in remediation of copper polluted red soils.

## 1 Materials and methods

### 1.1 Materials

#### 1.1.1 Soils

Surface layer (0–20 cm in depth) red soil samples from a paddy field were collected from Boli Village, Binjiang County, Guixi City, Jiangxi Province, China. Soils were air-dried, ground to pass through a 2 mm sieve, and homogenized. About 2.6 km<sup>2</sup> of paddy field have been contaminated with Cu and Cd by sewerage from an adjacent smelting factory (Jiangxi Copper Corporation, China) for more than 20 years (Hu et al., 2004). The total concentrations of Cu significantly exceed the environmental quality standard for agricultural soils (Cu 50 mg/kg in GB15618-1995) issued by the State Environmental Protection Administration of China. Research has shown that Cu pollution is the main reason for the reduction in rice output, which seriously affects the agricultural economy and the life quality of farmers (Li et al., 2008).

#### 1.1.2 Amendments

Attapulgite and montmorillonite clays, which are industrial products, were used as the soil amendments in this study. Attapulgite clay was supplied from Nanjing Yadong Aotu Mining Co., Ltd., China. Montmorillonite clay was obtained from the Zhejiang Fenghong Clay Chemicals Co., Ltd., China. The amendments were air-dried and ground to pass through a 0.049 mm mesh sieve.

#### 1.1.3 Sample analysis

The pH was measured using 0.1 mol/L of KCl (soil:solution ratio = 1:2.5) with a combination pH electrode (E-201-C, Shanghai Truelab Instrument Company, China). Cation-exchange capacity was detected using the acetate exchange method (Rhoades, 1982). Approximately, 1.0 g of homogenized soil samples were digested with nitric acid-perchloric acid, the samples were heated until the color became clear, dissolved with several drops of 1% HNO<sub>3</sub>, filtered, diluted to a volume of 25 mL with

double deionized water (DDW), and analyzed for the total content of Cu. Metal concentrations were determined by inductively coupled plasma-atomic emission spectrometry (ICP-AES, IRIS Advantage, Thermo Electron, USA). Three blanks (only extractant) were run to determine background concentrations. Mineralogical composition of clay minerals and soils was analyzed by means of X-ray diffraction (XRD, D/max-r B type X-ray diffraction instrument, Japan) with CuK $\alpha$  radiation.

### 1.2 Methods

#### 1.2.1 Copper adsorption experiments

All sorption studies were carried out in 50 mL centrifuge tubes by subjecting a 0.500 g sorbent to a shaking period of 24 hr with 20 mL of CuSO<sub>4</sub> solution on a vertical rotary shaker (150 r/min) at (25  $\pm$  1) $^{\circ}$ C. The initial metal concentration ranged from 1 to 100 mg/L, using 0.10 mol/L of NaOH or HCl solution to adjust pH to 5, and using 0.10 mol/L of KCl solution to adjust the ionic strength to 0.01 mol/L. The sorbent was separated using a centrifugation step (6000 r/min) over 10 min. The resulting solids were washed three times with 20 mL of 0.01 mol/L KCl solution and the centrifugal supernatant was stored at 4 $^{\circ}$ C. Metal concentrations in the supernatant were determined by atomic absorption spectrometry (AAS, Varian SpectrAA-220, USA). All assays were carried out in triplicates.

#### 1.2.2 Incubation experiments

Incubation experiments with clay minerals at different incubation times and the sequential extraction procedure were used to determine changes in heavy metal availability in the Cu contaminated soils. Nine treatments were run for each soil: untreated control soil, soil amended with 1% CM (clay minerals), soil amended with 2% CM, soil amended with 4% CM, and soil amended with 8% CM. Soil (600 g) was thoroughly mixed with the clays and was placed in a 250 mL incubation vessel. Double deionized water was added to the soils to increase moisture content to 70% of water-holding capacity. The incubations were carried out in triplicates, in a temperature-controlled incubator at (25  $\pm$  2) $^{\circ}$ C. After 30 and 60 days of incubation, the heavy metals of the soils were fractionated by a sequential extraction procedure. Chemical forms of heavy metals (Cu and Zn) were studied using the BCR sequential extraction procedure (Davidson et al., 1994). The heavy metal sequential extraction procedure was as follows: (1) acid exchangeable (EX): 1 g of dry soil extracted with 0.1 mol/L of CH<sub>3</sub>COOH (1:40, W/V) for 16 hr; (2) Fe and Mn oxides-bound (OX): residue from acid exchangeable fraction extracted with 0.1 mol/L of NH<sub>2</sub>OH-HCl (1:40, W/V) for 16 hr; (3) organic-bound (OB): residue from Fe and Mn oxides-bound fraction extracted with 30% H<sub>2</sub>O<sub>2</sub> (1:10, W/V) for 1 hr at 25 $^{\circ}$ C, 30% H<sub>2</sub>O<sub>2</sub> was added and mixture shaken for 1 hr at 85 $^{\circ}$ C, and then 1 mol/L CH<sub>3</sub>COONH<sub>4</sub> was added and mixture shaken for 16 hr at 25 $^{\circ}$ C; (4) residual forms (RES): residue from organic matter-bound fraction was removed into beakers, digested with aqua regia solution.

### 1.2.3 Biototoxicity experiments

Earthworms (*Eisenia foetida*) were provided by Nanjing Breeding Farms (China) and were cultured for three weeks before they were used. Healthy earthworms with annulus and of 200 mg weight were chosen. The experiment adhered to the artificial soil test following OECD guideline No. 207. Earthworms were cleaned and put in darkness for 24 hr before they were used. Nine treatments were run for each soil: control soil, soil amended with 1% CM, soil amended with 2% CM, soil amended with 4% CM, and soil amended with 8% CM. Soil was thoroughly mixed with the clays in accordance with the experimental design, and placed in a 1000 mL incubation vessel. After incubation for 48 hr, 10 earthworms were put in each vessel, which were covered by gauze to prevent escape. Double deionized water was added to the soils to increase moisture content to 80% of water-holding capacity. The incubations were carried out in triplicate, in a temperature-controlled incubator at  $(20 \pm 2)^\circ\text{C}$ . After two weeks incubation, poisoning symptoms and mortality of the earthworms were observed. Earthworms were cleaned, put in darkness for 24 hr and air dried in natural conditions. Protein content was detected with the Coomassie Brilliant Blue method (Bradford et al., 1976).

### 1.3 Statistical analysis

Using SPSS Version 13.0 software (SPSS Inc., USA), data were subjected to ANOVA and differences between means were determined using the Duncan test. Correlation analysis was conducted using the Pearson method. In each figure, the  $p$  value included was the ANOVA result on the whole data sets,  $p < 0.05$  and  $p < 0.01$  mean significant differences and extremely significant differences, respectively.

## 2 Results and discussion

Selected physiochemical properties and the concentrations of some elements of the amendments are given in Table 1.

**Table 1** Basic properties of montmorillonite, attapulgite, and soil

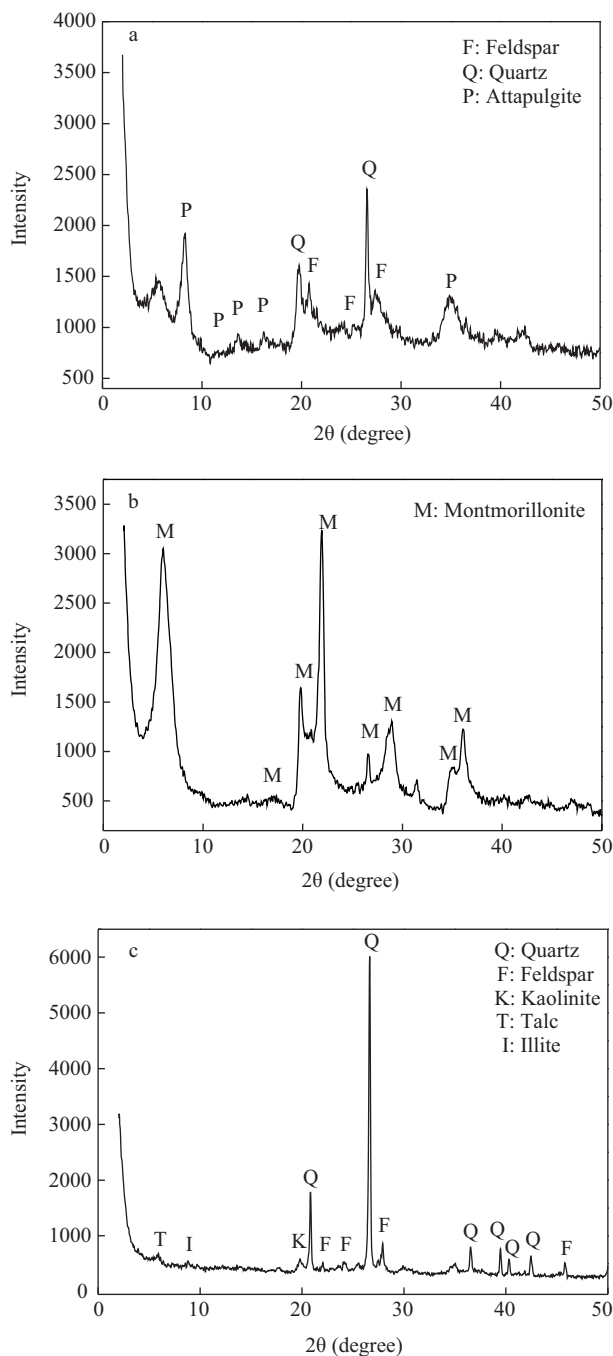
Sample	pH	CEC (cmol/kg)	Total heavy metal content (mg/kg)		
			Cu	Zn	Cd
Attapulgite	7.12	28.5	18.6	5.53	0.57
Montmorillonite	8.5	38.4	4.61	6.68	0.773
Soil	3.99	9.29	547	70.1	1.93

CEC: cation exchange capacity.

Critical values of the soil environmental quality standard in China (GB 15168-1995) were total Cd 0.3 mg/kg, total Zn 200 mg/kg and total Cu 50 mg/kg for red soil (pH < 6.5).

### 2.1 XRD analysis of attapulgite, montmorillonite and soil

X-ray diffraction (XRD) patterns are shown in Fig. 1. Attapulgite showed good crystallinity, with purity greater than 80% and a low amount of quartz and feldspar. Mont-

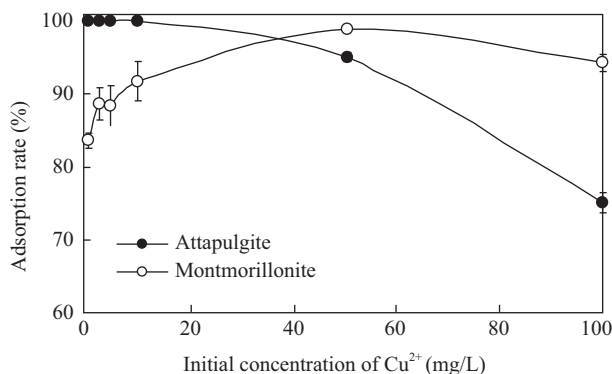


**Fig. 1** XRD patterns of attapulgite (a), montmorillonite (b), and soil (c).

morillonite also showed good crystallinity, with purity greater than 90%. Polluted red soil was mainly composed of quartz, with a small amount of feldspar, kaolinite, talc, and illite.

### 2.2 Copper adsorption and desorption experiments

Attapulgite and montmorillonite displayed good adsorption for  $\text{Cu}^{2+}$ . The adsorption rate of  $\text{Cu}^{2+}$  from 1 to 10 mg/L of copper sulphate solution on attapulgite was 100%, when the concentration was over 50 mg/L, the adsorption rate reduced with increasing initial concentration of copper. The maximum adsorbed  $\text{Cu}^{2+}$  by attapulgite and montmorillonite was 1501 and 3741 mg/kg, respectively (Fig. 2).



**Fig. 2** Relationship between the adsorption rate and initial concentration of  $\text{Cu}^{2+}$ .

The desorption rate can be used to characterize the degree of  $\text{Cu}^{2+}$  binding to the clay. The higher the percentage of desorption, the more loose the binding. The results showed that montmorillonite displayed a higher desorption amount than attapulgite. Copper on attapulgite was 100% fixed with four concentration points, while copper on montmorillonite was desorbed a little at all concentration points (Table 2). When desorbed three times, no significant difference was observed between the two CM on sorption amount. When the initial Cu concentration reached 50 and 100 mg/L, the adsorption amounts for montmorillonite were 1826.4 and 3359.5 mg/kg respectively, which were much higher than the adsorption amount for attapulgite. Morton et al. (2000) reported that the adsorption reversibility of copper surface-associations on the permanent charge and edge sites of montmorillonite can be explained by either the formation of irreversible multinuclear complexes on the edge sites of clay or by the fact that different types (aluminol vs. silanol) or locations (bulk vs. surface hydroxyl) of sites found on the edges of clay have different affinities for copper and reversibility. Given the hydrated structure and weak association evidenced by the ability of background electrolyte cations to displace interlayer metal cations, it is not surprising that the outer-sphere complexes formed by copper on these sites are reversible (Morton et al., 2000). Thus, the adsorption of Cu on attapulgite was irreversible at firstly, owing to Cu complexes with attapulgite may be fixed by the different types of active sites with high oxide content. After the adsorption

**Table 3** Effects of attapulgite and montmorillonite on soil pH

Clay mineral	Addition amount (%)	Soil pH	
		30-day incubation	60-day incubation
Control	0	4.06 a	3.97 a
Attapulgite	1	4.14 c	4.27 c
	2	4.16 cd	4.30 c
	4	4.23 e	4.37 d
	8	4.35 f	4.41 de
Montmorillonite	1	4.43 g	4.45 e
	2	4.69 h	4.64 f
	4	4.95 i	4.60 f
	8	5.18 j	4.79 g

amount of Cu increased to some extent, a part of Cu which adsorbed on the permanent negative charge surface could be desorbed. The specific adsorption of Cu in low concentration on attapulgite plays an important role in the control of Cu concentration in soil solutions. Adsorption of Cu on montmorillonite was both irreversible and reversible at the same time, mainly in ion exchange. In relation to environmental protection, the interaction between CM and heavy metals are meaningful.

### 2.3 Indoor incubation experiments

#### 2.3.1 Effects of attapulgite and montmorillonite on soil pH

The polluted soil was acidic (pH 3.99). When cultured over 30 and 60 days after attapulgite and montmorillonite addition, soil pH increased significantly compared to the control (Table 3). As the addition amount ranged from 1% to 8%, the soil pH increased with increasing attapulgite and montmorillonite dose. Montmorillonite promoted the greatest increase (0.37–1.12 pH increased) in soil pH, compared to attapulgite. Soil pH plays an important role in bioavailability of heavy metals. An increase in pH affects metal cations retention, leading to a reduction in bioavailability of heavy metals. Due to the formation of  $\text{OH}^-$  complexes, the mobility of Cu is usually slowest in a slightly alkaline soil but it can increase in a highly alkaline soil (pH > 10). In this study, although application of attapulgite and montmorillonite altered soil pH, it was still within the range of soil pH (4.02–5.18) for efficient metal immobilization (Chen et al., 2007).

**Table 2** Copper desorbed from attapulgite and montmorillonite

Clay mineral	Initial conc. (mg/L)	Adsorption (mg/kg)	Desorption						Residual $\text{Cu}^{2+}$ amount	
			Desorption (mg/kg)	Desorption/adsorption (%)	Desorption (mg/kg)	Desorption/adsorption (%)	Desorption (mg/kg)	Desorption/adsorption (%)	Residual (mg/kg)	Residual/adsorption (%)
Attapulgite	1	40.1	0	0	0	0	0	0	40.1	100
	3	119	0	0	0	0	0	0	119	100
	5	198	0	0	0	0	0	0	198	100
	10	399	0	0	0	0	0	0	399	100
	50	951	7.75	0.82	0.09	0.01	0	0	943	99.2
	100	1501	132	8.80	22.0	1.47	2.76	0.18	1344	89.5
Montmorillonite	1	33.7	2.52	7.47	1.60	4.87	0	0	29.5	87.7
	3	107	3.52	3.28	0.13	0.13	0	0	104	96.6
	5	177	12.9	7.29	2.00	1.13	0	0	162	91.6
	10	368	21.2	5.76	3.80	1.03	0	0	343	93.2
	50	1961	121	6.17	6.42	0.33	7.40	0.38	1826	93.1
	100	3741	265	7.08	70.3	1.88	46.3	1.24	3359	89.8

### 2.3.2 Chemical speciation of Cu in soil

In regards to heavy metal distribution and morphological characteristics, Ma and Uren (1998) reported that pollutants from smelters were mainly in a more soluble form (such as acid exchangeable phase), and were oxidized to relatively non-active states (such as oxides-bound phase). When the acid exchangeable phase was generally higher than the residual phase in soil, it was considered to be an effect from man-made sources. Distribution of Cu in the arable soil from this study showed the effect of man-made sources. In China the background levels of Cu content in red soils (Ferralsols) typically vary from 15 to 155 mg/kg, with a mean value of 41 mg/kg (Kabata-Pendias and Pendias, 2001). Total Cu content in the polluted soil from our study was 547 mg/kg, which is higher than the environmental quality standard for Cu in agricultural soils in China and 9.76 times higher than average background values. Copper in the treatments without amendments was primarily associated with bound and residual phases, followed by acid exchangeable + organic-bound + Fe and Al oxides-bound  $\gg$  residual. Acid exchangeable fractions accounted for 32.7%–44.8% for total Cu. Residual Cu content in the soil was 14.5–17.5 mg/kg (2.65%–3.20%), which was significantly lower than that in normal soil (50%–75%) (Ma et al., 1992), indicating that bioavailability and the environmental risk of Cu is higher.

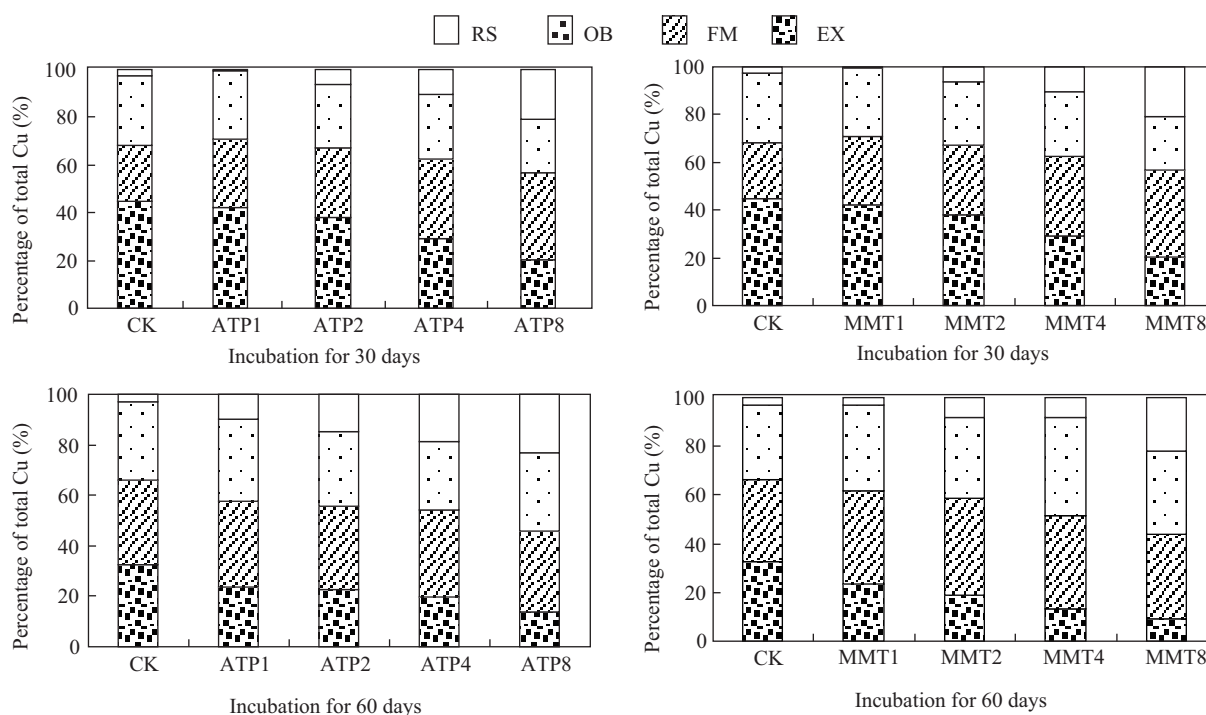
Figure 3 shows the distribution of Cu as assessed by the sequential extraction method in the soils. As the addition amount increased from 1% to 8%, acid exchangeable Cu decreased from 18.75% to 5.09% for attapulgite amended soil and from 24.74% to 2.46% for montmorillonite amended soil. These results showed that the remediation effect of acid exchangeable Cu is montmorillonite > at-

tapulgite. When montmorillonite was increased in soil by 8% after incubation for 30 days, acid exchangeable Cu decreased by 24.7% compared to the control. Acid exchangeable Cu decreased with increasing montmorillonite amount, with the best remediation effect observed at an 8% dose.

Addition of CM did not significantly change Cu in organic-bound and Fe and Mn oxides-bound phases. As the addition amount increased from 1% to 8%, Fe and Mn oxides-bound Cu increased from 1.67% to 6.23% and from 0.98% to 13.07% for attapulgite and montmorillonite amended soil, respectively. Organic-bound Cu decreased from 11.49% to 0.41% and from 9.52% to 6.66% for attapulgite and montmorillonite amended soil, respectively.

Residual Cu in the treatments with clay minerals significantly increased (Fig. 2). Previous work has reported residual heavy metal levels in mildly acidic or alkali soils were obviously higher than those in the neutral state, possibly as a result of higher soil pH and the dominance of 2:1 clay minerals in soils (Bodek et al., 1998). As the addition amount increased from 1% to 8%, residual Cu increased from 5.09% to 22.04% and from 0 to 18.75% for attapulgite and montmorillonite amended soil, respectively. With increasing addition amount, the residual Cu fraction also increased, reaching optimal values from 135.3 to 170.3 mg/kg (from 23.2% to 24.7%) for attapulgite at 8%.

As the incubation time was prolonged from 30 to 60 days, acid-extractable Cu of the control soil decreased from 44.8% to 32.7%, Fe-Mn oxides-bound Cu increased from 23.5% to 33.4%, while organic-bound and residual Cu showed no significant changes. The decreasing amount of acid-extractable Cu was similar to the increasing



**Fig. 3** Relative percentages of Cu in each fraction of the soils from the CK and clays treated soils (mean,  $n = 3$ ). EX: acid exchangeable; FM: Fe and Mn oxides-bound; OB: organic-bound; RS: residual; ATP: attapulgite; MMT: montmorillonite.

amount of Fe-Mn oxides-bound Cu. Thus, in the soil without amendments, reduction-oxidation reaction of Fe-Mn oxides occurred. These oxides dissolved or formed precipitates of compounds which makes a number of copper ions entrapped (Fig. 3). Acid-extractable Cu mainly changed into residual Cu. Acid-extractable Cu decreased and residual Cu increased with clay mineral addition, and had little effect on Fe-Mn oxides-bound and organic-bound Cu. It is obvious that clay minerals were beneficial for the reduction of Cu from high toxicity to low toxicity.

#### 2.4 Toxicity testing with earthworms

Earthworms (*Eisenia fetida*) were studied to determine the effect of Cu on the growth and body protein content, which provided a basis for biological diagnosis and risk assessment of heavy metal polluted soils. Copper induced fatality depends on soil properties and components. The LC<sub>50</sub> of European soil (643 mg/kg) (Neuhauser et al., 1985) is 1.5 times that of Chinese soil (400–450 mg/kg) (Song et al., 2002). Studies have shown that the increased soil pH can increase the adsorption of soil and reduce the biological toxicity of heavy metals (Oka et al., 2007). The higher the pH value, the easier the molecule is ionized and the smaller the molecular size proportion. Heavy metals cannot easily move from earthworm skin surface into their internal body as the biological toxicity of heavy metals reduced. Therefore, biological toxicity of heavy metals can be reduced by changing soil properties and components. Our experiments showed that the poisoning effect of Cu on earthworms was reduced with the addition of attapulgite and montmorillonite, with montmorillonite showing the best effect. With the addition of a 2% dose, mortality decreased from 60% to zero compared to the control. Toxic symptoms included body atrophy, fracture, bleeding, body softening, hyperemia of annulus, and yellow liquid exudation. Weight growth rate in all treatments was slower than the control, that is, there was a negative effect on growth rate (Table 4). This was likely caused by the toxic effect of Cu on earthworms. It should be noted that no food was provided to the earthworms during the incubation time, which may have also influenced growth rate. In addition, body protein content increased with the addition of attapulgite and montmorillonite, although the reason for this is unclear and merits further research.

**Table 4** Results of toxicity testing with earthworms

Clay mineral	Addition amount (%)	Growth rate (%)	Death rate (%)	Body protein content (g/L)
Control	0	–	60	2.40
Attapulgite	1	–	30	2.30
	2	–	10	3.18
	4	–0.015	0	3.11
	8	–0.052	0	2.02
Montmorillonite	1	–0.143	0	1.59
	2	–0.035	0	2.12
	4	–0.209	0	2.77
	8	–0.076	0	2.27

Growth rate was calculated with the following equation:  $r_g = (W_a - W_b) / W_b \times 100$ , where,  $r_g$  (%) is the growth rate;  $W_a$  (g) is the weight after incubation, and  $W_b$  (g) is the weight before incubation.

### 3 Conclusions

Attapulgite and montmorillonite were effective at remediation of heavy metal polluted soils. From the sorption experiment, the maximum adsorbed Cu<sup>2+</sup> by attapulgite and montmorillonite was 1501 and 3741 mg/kg, respectively. The adsorption of Cu on attapulgite was firstly irreversible, when the initial concentration of Cu<sup>2+</sup> reached 50 mg/kg, a part of Cu was adsorbed reversibly and could be desorbed. Adsorption of Cu on montmorillonite was both irreversible and reversible at the same time, mainly in ion exchange. After incubation for 30 and 60 days, soil pH was significantly increased. A significant mitigative effect for attapulgite and montmorillonite on Cu polluted soils was observed, with montmorillonite displaying a better effect than attapulgite. The results of distribution of Cu showed that acid-extractable Cu decreased and residual Cu increased for attapulgite and montmorillonite amended soils. The poisoning effect of Cu on earthworms was reduced with the addition of attapulgite and montmorillonite, with montmorillonite showing the best effect. With the addition of a 2% dose, mortality decreased from 60% to zero compared to the control.

China has rich reserves of inexpensive clay minerals. They show good prospects for development in remediation of heavy metal polluted soils. Because of differences between indoor experiments and natural soil conditions, future field tests are needed to clarify soil remediation effects under natural conditions and to determine proper addition quantities.

#### Acknowledgments

This work was supported by the National Basic Research and Development (973) Program of China (No. 2007CB936604) and the Knowledge Innovation Program of the Chinese Academy of Sciences (No. KSCX2-YW-N-038). Thanks are given to Assistant Engineer Hua Gong and Senior Engineer Jie Chen from the Institute of Soil Science, Chinese Academy of Sciences, Nanjing, China, for their assistance with sample analysis.

#### References

- Bradford M M, 1976. A rapid and sensitive method for the quantitation of microgram of protein utilizing the principle of protein dye binding. *Analytical Biochemistry*, 72: 248–254.
- Bodek I, Lyman W, Reehl W F, Rosenblatt D H, 1988. *Environmental Inorganic Chemistry*. Pergamon Press, New York.
- Cao X D, Wahbi A, Ma L N, Li B, Yang Y L, 2009. Immobilization of Zn, Cu, and Pb in contaminated soils using phosphate rock and phosphoric acid. *Journal of Hazardous Materials*, 164: 555–564.
- Celis R, Hermosin M C, Cornejo J, 2000. Heavy metal adsorption by functionalised clays. *Environmental Science and Technology*, 34: 4593–4599.
- Chen S B, Xu M G, Ma Y B, Yang J C, 2007. Evaluation of different phosphate amendments on availability of metals in contaminated soil. *Ecotoxicology and Environmental Safety*, 67: 278–285.

- Cheng S F, Hseu Z Y, 2002. *In situ* immobilization of cadmium and lead by different amendments in two contaminated soils. *Water Air and Soil Pollution*, 140: 73–84.
- Churchman G J, 2002. The role of clays in the restoration of perturbed ecosystems. *Developments in Soil Science*, 28: 333–350.
- Davidson C M, Thomas R P, McVey S E, Perala R, Littlejohn D, Ure A M, 1994. Evaluation of a sequential extraction procedure for the speciation of heavy metals in sediments. *Analytica Chimica Acta*, 291: 277–286.
- Geebelen W, Vangronsveld J, Adriano D C, Carleer R, Clijsters H, 2002. Amendment-induced immobilization of lead in lead-spiked soil: Evidence from phytotoxicity studies. *Water Air and Soil Pollution*, 140: 261–277.
- Gray C W, Dunham S J, Dennis P G, Zhao F J, McGrath S P, 2006. Field evaluation of *in situ* remediation of a heavy metal contaminated soil using lime and red-mud. *Environmental Pollution*, 142: 530–539.
- Hu N J, Li Z Q, Huang P, Tao C, 2004. Chemical forms of heavy metals in sewage-irrigated paddy soil in Guixi City. *Journal of Agro-Environment Science*, 23: 683–686.
- Kabata-Pendias A, Pendias H, 2001. Trace Elements in Soils and Plants (3rd ed.) CRC Press, New York.
- Kim Y, Kim J H, Lee K G, Kang S G, 2005. Adsorption behavior of heavy metal ions in the solutions of clay minerals under various conditions. *Journal of Ceramic Processing Research*, 6: 25–30.
- Li P, Wang X X, Zhang T L, Zhou D M, He Y Q, 2008. Effects of several amendments on rice growth and uptake of copper and cadmium from a contaminated soil. *Journal of Environmental Sciences*, 20(4): 449–455.
- Liu L N, Chen H S, Cai P, Liang W, Huang Q Y, 2009. Immobilization and phytotoxicity of Cd in contaminated soil amended with chicken manure compost. *Journal of Hazardous Materials*, 163: 563–567.
- Ma Y B, Xia R J, Zhang G Y, Sun Z Y, 1992. Chemical forms of soil Cu in Hebei Province. *Acta Agriculture Universitatis Pekinensis*, 18: 33–38.
- Ma Y B, Uren N C, 1998. Transformations of heavy metals added to soils – Application of a new sequential extraction procedure. *Geoderma*, 84: 157–168.
- McBride M B, Martínez C E, 2000. Copper phytotoxicity in a contaminated soil: Remediation tests with adsorptive materials. *Environmental Science and Technology*, 34: 4386–4391.
- Morton J D, Semrau J D, Hayes K F, 2000. Structure and reversibility of copper adsorption to montmorillonite clay, In: Chemical Speciation and Reactivity in Water Chemistry and Water Technology: A Symposium in Honor of James (Morgan J, organized by Hering J and Schnoor J L). American Chemical Society, Washington DC, USA. 650–652.
- Narwal R P, Singh B R, 1998. Effect of organic materials on partitioning, extractability and plant uptake of metals in an alum shale soil. *Water Air and Soil Pollution*, 103: 405–421.
- Neuhauser E F, Loehr R C, Milligan D L, Malecki M R, 1985. Toxicity of metals to the earthworms *Eisenia fetida*. *Biology and Fertility of Soils*, 1: 149–152.
- Oka Y S, Yang J E, Zhang Y S, 2007. Heavy metal adsorption by a formulated zeolite – Portland cement mixture. *Journal of Hazardous Materials*, 147: 91–96.
- Pandian N S, Sridharan A, Rajasekhar C, 2001. Heavy metal retention behavior of clayey soils. *Journal of Test and Evaluation*, 29: 361–371.
- Querol X, Alastuey A, Noreno N, Alvarez-Ayuso E, Garcia-Sanchez A, Camal J et al., 2006. Immobilization of heavy metals in polluted soils by the addition of zeolitic material synthesized from coal fly ash. *Chemosphere*, 62: 171–180.
- Rhoades J D, 1982. Cation exchange capacity. In: Methods of Soil Analysis, Part 2 (Page A L, Miller R H, Keeney D R, eds.) (2nd ed.). Agronomy Monographs 9, ASA and SSSA, Madison, WI. 149–157.
- Song Y F, Zhou Q X, Xu H X, Ren L P, Sun T H, Gong P, 2002. Acute toxicological effects of heavy metal pollution in soils on earthworms. *Chinese Journal of Applied Ecology*, 13: 187–190.
- Toribio M, Romanyà J, 2006. Leaching of heavy metals (Cu, Ni and Zn) and organic matter after sewage sludge application to Mediterranean forest soils. *Science of the Total Environment*, 363: 11–21.
- Vangronsveld J, Cunningham S D, 1998. Metal-contaminated Soils: *In Situ* Inactivation and Phytoremediation. Springer-Verlag Berlin Heidelberg and R G Landes Company, Georgetown, TX.