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Flooding-enhanced immobilization effect of sepiolite on cadmium in paddy soil

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Abstract

Purpose Little is known of the effect of sepiolite on the transformation of Cd in anthropogenically contaminated paddy soil under different moisture conditions; therefore, we studied the effects of sepiolite and flooding on the extractability and fractionation of Cd in paddy soils.

Materials and methods The dynamics of soil Eh, pH, DTPA-extractable Cd, and different Cd soil fractions were studied in two typical paddy soils from south China that were spiked with 10 mg kg^{-1} Cd following amendment with sepiolite at 5 and 10 gkg^{-1} soil during a 30-day incubation period at 25°C, with either no flooding or continuous flooding conditions.

Results and discussion The addition of sepiolite at two rates of 5 and 10 gkg^{-1} soil resulted in an average reduction in soil Eh of 76 and 93 mV, increase in soil pH of 1.2 and 2.3 pH units, and decrease in DTPA-extractable Cd in soils of 1.43 and 2.53 mg kg^{-1} under continuous flooding conditions, respectively. Sepiolite addition resulted in a significant decrease in the exchangeable Cd in the soils, and a significant increase, in the carbonate-bound and Fe/Mn oxide-bound Cd in the soils under both moisture conditions. Cadmium was also immobilized by flooding and by interactions between sepiolite application and flooding; these effects were greater in sandy paddy soil than in clay paddy soil.

Conclusions The immobilization of Cd in typical paddy soils was related mainly to changes in Eh and pH caused by the addition of sepiolite and flooding. Sepiolite can be used in the remediation of Cd-contaminated paddy soils, especially in sandy paddy soils, and flooding enhances the stabilization of Cd in paddy soils by sepiolite.

Keywords Cadmium · Extractability · Flooding · Fractionation · Paddy soil · Sepiolite

1 Introduction

Elevated Cd levels in agricultural soils, resulting from mining activities, industrial emissions, and the application of sewage sludge or phosphorus fertilizer, are becoming a major environmental problem due to its great toxicity and high mobility from soil to plants and thereby into the food chain (Wang and Xing 2002; Wei et al. 2009). In China, the national rice yield of 2009 was 1.95×10^{8} t year^{-1} which constitutes about 40% of the national grain yield (4.82×10^{8} t year^{-1}), and rice is a staple food for more than 60% of the population (National Bureau of Statistics of China 2010; Yang et al. 2006). Approximately 2.8×10^{5} hm^{2} of farmland in China is contaminated with Cd (State Environmental Protection Agency 2003). The maximum Cd content in contaminated paddy field varies from 5.0 to 145.0 mg kg^{-1}, resulting in the maximum accumulation of Cd in brown rice at levels from 1.9 to 9.4 mg kg^{-1} (Zhang 2008), thereby

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posing a risk to human and animal health (Jalloh et al. 2009). However, the maintenance of Cd levels within physiological ranges in plants does not depend exclusively on the total soil Cd concentration it also depends on the distribution of Cd among various chemical fractions (Chen et al. 2000). In China, rice is generally cultured by rotation through flooded and unflooded conditions to meet its needs for growth. The submergence of soils has been reported to cause the redistribution of Cd into different chemical fractions and to reduce the availability of Cd in soils. This reduce of availability is attributed to the increased adsorption of the metal to hydrous Mn and Fe oxides and to the formation of insoluble CdS (Kashem and Singh 2001; Sun et al. 2007).

Moreover, the bioavailability and mobility of Cd in soils can be reduced by chemical and biological immobilization (Hong et al. 2008). Recently, there has been keen interest in the immobilization of heavy metals using clay minerals, such as sepiolite (Xu et al. 2007; Zhu et al. 2010), bentonite (Cheng and Hseu 2002; van Herwijnen et al. 2007), montmorillonite (Badora et al. 1998), and Penghu soil (Cheng and Hseu 2002). Sepiolite is a layered silicate clay mineral found in many soils in arid and semiarid regions, in association with carbonates or quartz (Özdemir et al. 2007). It has a ribbon-like structure formed from two inverted silica tetrahedral sheets with a magnesium octahedral sheet between them, producing alternating hollow channels that allow the penetration of the structure by solutes. Some isomorphic substitutions in the tetrahedral layer, such as Al^{3+} for Si^{4+}, also produce negatively charged adsorption sites that can electrostatically adsorb cations (Xu and Hseung 1983). Previous studies have reported that sepiolite has a high sorption capacity for Cd and the ability to reduce the mobility of Cd in soils polluted by mining activities (Álvarez-Ayuso and García-Sánchez 2003; Shirvani et al. 2006a, b). Consequently, sepiolite has been recommended for the remediation of Cd-contaminated paddy soils (Zhu et al. 2010).

However, few studies have examined the effects of sepiolite on the transformation of Cd in artificially contaminated paddy soil under different moisture conditions. Therefore, the objective of this study was to investigate the extractability and fractionation of Cd in soils in relation to changes in the soil properties caused by the application of sepiolite and continuous flooding.

2 Materials and methods

2.1 Soil and sepiolite preparation

A sepiolite sample (particle diameter ≤74 μm) was purchased from Hongyan Ltd. (Xiangtan, Hunan Province, China), consisting of sepiolite (mainly as $Mg_4SiO_{15}(OH)_2 \cdot H_2O$ and $2MgOSiO_2 \cdot H_2O$) and accessory minerals such as quartz, as observed by X-ray diffraction. A sample of clayey paddy soil (0–20 cm depth) derived from Quaternary red clay, classified as reddish yellow clayey paddy soil (RY; Chinese Soil Taxonomy), and a sample of sandy paddy soil (0–20 cm depth) derived from arenaceous shale, classified as reddish sandy paddy soil (RS; Chinese Soil Taxonomy), were collected from Taoyuan County, Hunan Province, China. The selected properties of the soils [e.g., soil pH, organic carbon, cation exchange capacity (CEC), texture, total Cd] were measured using routine analytical methods and are listed in Table 1. After collection, the soils were air-dried, ground, and passed through a 2-mm mesh sieve. Cadmium (10 mg kg^{-1} soil, as a $CdCl_2$ solution) was added to each soil sample (7,200 g), and the samples were mixed thoroughly. Next, deionized water was added to soil during mixing until the level of standing water was at a height of 2.0±0.5 cm above the soil surface (flooding). The flooded soil samples were then incubated at room temperature (25±1°C), and the soil water was added to the flooding level every 2 days. After a 7-day incubation, the soils were air-dried and rehomogenized by sieving.

2.2 Incubation

For each soil, we employed a 2×3 factorial design with two levels of moisture condition and three levels of sepiolite addition, making a total of six treatments, denoted NF0 [no flooding (45% water holding capacity (WHC)) with sepiolite added at 0 gkg^{-1} soil], NF5 (no flooding with sepiolite added at 5 gkg^{-1} soil), NF10 (no flooding with sepiolite added at 10 gkg^{-1} soil), F0 (continuous flooding with sepiolite added at 0 gkg^{-1} soil), F5 (continuous flooding with sepiolite added at 5 gkg^{-1} soil), and F10 (continuous flooding with sepiolite added at 10 gkg^{-1} soil). Four replicates of each treatment were tested. Each subsample (300 g) was placed in an open plastic jar (diameter 13 cm×height 15 cm). The jars were

Table 1 Basic properties of the two paddy soils studied

Soil	pH	SOC (g kg^{-1})	TN	TP	Olsen P (mg kg^{-1})	AK	CEC (cmol (+) kg^{-1})	Sand (%)	Silt	Clay	TCd (mg kg^{-1})
RY	5.0	17.9	2.20	1.24	18.1	83	13.3	21.6	50.8	27.6	ND
RS	5.1	16.1	1.7	0.68	27.4	89	11.8	59.6	22.0	18.4	ND

RY reddish yellow clayey paddy soil, *RS* reddish sandy paddy soil, *SOC* soil organic carbon, *TN* total nitrogen, *TP* total phosphorus, *AK* available K, *TCd* total cadmium, *ND* not detectable placed in polypropylene barrels (100 L) with 500 mL of water to maintain 100% humidity. The barrels were sealed, and the soil samples were incubated at 25±1°C for 30 days. In continuous flooding culture, deionized water was added to soil during mixing until the level of standing water was at a height of 2.0±0.5 cm above the soil surface, whereas in no flooding culture the soil moisture was adjusted to 45±5% of WHC and both cultures were maintained by periodic (every 2 days) addition of deionized water throughout the 30-day incubation period. After 2, 5, 10, 15, 20, and 30 days, the barrels were opened and oxidation–reduction potential (Eh) of soil was determined and a subsample was taken from each jar to measure the amount of diethylenetriaminepentaacetic acid (DTPA)-extractable Cd (Lindsay and Norvell 1978) in the soil and soil pH. For the subsamples tested at the end of incubation (day 30), the Cd soil fractions present in the soil were determined with sequential extraction (Tessier et al. 1979).

2.3 Analysis

The DTPA extraction method is described briefly: 20.00 g of soil in 40 mL of 0.05 M DTPA+0.01 M $CaCl_2$+0.01 M triethylolamine (TEA, pH 7.3) was shaken for 2 h. The sequential extraction method of Tessier et al. (1979) was performed in five steps, assuming that the Cd soil fractions extracted were as follows:

1. Fraction 1 (water soluble plus exchangeable, EX-Cd): 1.000 g of soil (dry weight) was extracted with 8 mL of 1 M $MgCl_2$ solution and shaken for 1 h at 25±1°C, and the extract was separated from the solid residue by centrifugation at 3,000×*g* for 20 min.
2. Fraction 2 (carbonate bound, CB-Cd): to the fraction 1 residue was added 8 mL of 1 M CH_3COONa solution (pH 5, adjusted with acetic acid), which was shaken for 5 h at 25±1°C; the extract was then centrifuged as for fraction 1.
3. Fraction 3 (Fe/Mn oxide bound, OX-Cd): to the fraction 2 residue was added 20 mL of 0.04 M $NH_2OH{\cdot}HCl$ in 25% acetic acid. The mixture was refluxed at 96±3°C with occasional agitation for 6 h, and then the extract was centrifuged as for fraction 1.
4. Fraction 4 (organic matter bound, OC-Cd): to the fraction 3 residue were added 3 mL of 0.02 M HNO_3 and 5 mL of 30% H_2O_2, and the pH was adjusted to 2.0 with HNO_3. The mixture was refluxed at 85±2°C for 2 h. A second 5-mL aliquot of 30% H_2O_2 (pH 2) was added, and the sample was again refluxed for 3 h. After cooling, 5 mL of 3.2 M ammonium acetate in 20% (*v*/*v*) HNO_3 was added. The sample was diluted to 20 mL and agitated continuously for 30 min, and then the extract was centrifuged as for fraction 1.
5. Fraction 5 (residual, RES-Cd): the residue of fraction 4 was digested (open system) using a mixture of 15 mL aqua regia and 3 mL of $HClO_4$.

The Cd concentrations in the solutions were determined by atomic absorption spectroscopy (AAS; GBC, Australia). Organic C and total N in soil were measured by dry combustion in a CN auto-analyser (Vario MAX C/N, Germany), total P was measured by the NaOH fusion method (Olsen and Somers 1982), and Olsen P and available K were extracted with 0.5 M $NaHCO_3$ and 1 M CH_3COONH_4, respectively. The CEC was determined with the ammonium acetate method (Chapman 1965). Soil texture (sand, silt, and clay) was determined with the hydrometer method (Gee and Bauder 1986). The total content of Cd in soil was determined with aqua regia and $HClO_4$ digest. Briefly, 1.000 g soil samples (dried weight) were digested (open system) using a mixture of 20 mL aqua regia and 3 mL $HClO_4$, and concentrations of Cd in digested solution were determined by AAS for the total content of Cd. Three certified reference materials, GBW070011 Chinese soil samples, three spikes and three blanks were used for quality control. Soil Eh was measured during incubation using an ORP combination Pt-band electrode (ORP431; Shanghai Dapu Instrument, China). The soil and sepiolite pH was determined in water at a soil-to-solution ratio of 1:2.5 (*w*/*v*) using a pH meter (PHS-3C; Shanghai Dapu Instrument, China). Analysis of variance was used to compare means, employing SPSS statistical software (SPSS 11.5 system for Windows).

3 Results

3.1 Effect of sepiolite and flooding on soil Eh, pH, and DTPA-extractable Cd in soil

3.1.1 No flooding soils

For the no flooding treatments, soil Eh ranged from 242 to 355 mV and from 227 to 376 mV for soils RY and RS, respectively (Fig. 1). The addition of sepiolite significantly ($p<0.01$) decreased soil Eh by about 47 and 80 mV on average after the NF5 and NF10 treatments, respectively. Moreover, during the 30-day incubation period, the soil Eh remained essentially constant for the three no flooding treatments of both soils. Therefore, the differences in the soil Eh between the NF0, NF5, and NF10 treatments remained generally constant throughout the 30-day incubation period in both soils.

However, the addition of sepiolite significantly ($p<0.01$) increased soil pH by about 0.9 and 2.0 pH units on average for the NF5 and NF10 treatments in soil RY, and by about

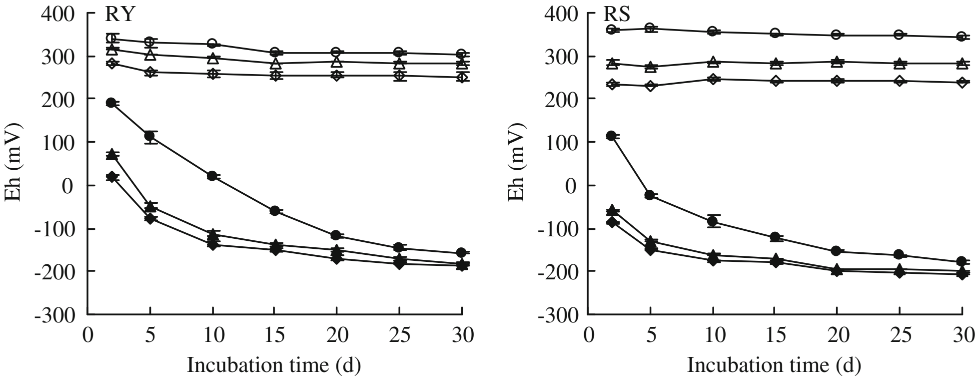


Fig. 1 Changes in soil Eh with and without sepiolite under different moisture conditions. NF0, no sepiolite applied with no flooding (○); NF5, sepiolite applied at 5.0 gkg^{-1} soil with no flooding (△); NF10, sepiolite applied at 10.0 gkg^{-1} soil with no flooding (◇); F0, no sepiolite applied with continuous flooding (●); F5, sepiolite applied at 5.0 gkg^{-1} soil with continuous flooding (▲); F10, sepiolite applied at 10.0 gkg^{-1} soil with continuous flooding (◆). *RY* reddish yellow clayed paddy soil, *RS* reddish sandy paddy soil. *Bars* indicate the standard deviation of the mean

1.6 and 2.6 pH units on average for the NF5 and NF10 treatments in soil RS, respectively (Fig. 2). During the 30-day incubation period, the soil pH of both soils, treated and untreated with sepiolite, showed a gradual decline. The differences in soil pH of NF5, NF10, and NF0 were gradually reducing during the incubation period.

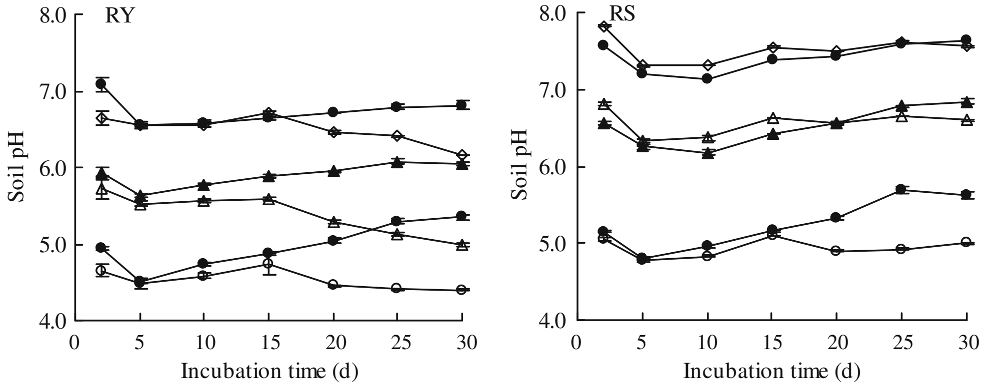
The amount of DTPA-extractable Cd in soil showed a significant decrease ($p<0.01$) with sepiolite addition, by 0.26 and 0.85 mg kg^{-1} for the NF5 and NF10 treatments in soil RY, and by 1.76 and 3.90 mg kg^{-1} for the NF5 and NF10 treatments in soil RS, respectively (Fig. 3). During the 30-day incubation period, DTPA-extractable Cd in both soils, treated and untreated with sepiolite, showed a steady increase. By the end of incubation period, the amount of soil DTPA-extractable Cd had increased by 0.51–1.51 mg kg^{-1} compared with the values on day 2. For soil RY, the differences in DTPA-extractable Cd between sepiolite-treated soils (NF5 and NF10) and untreated soils (NF0) declined continuously during days 2 to 30. While, for soil RS, these differences remained generally constant throughout the incubation period and were much larger than these for soil RY.

3.1.2 Continuous flooding soils

For both soils under continuous flooding condition, the effect of sepiolite on soil Eh was similar to that in the no flooding soils (see Fig. 1). The addition of sepiolite significantly ($p<0.01$) decreased the soil Eh by 76 and 93 mV on average for the F5 and F10 treatments, respectively. However, the soil Eh of sepiolite-treated and untreated soils (F0, F5, and F10) showed a sharp decrease by 206–348 mV and 119–292 mV for soils RY and RS, respectively, during days 2 to 30. Moreover, the differences in soil Eh between sepiolite-treated soils (F5 and F10) and untreated soil F0 markedly reduced during the incubation period.

Soil pH, as measured in continuous flooding soils, ranged from 4.5 to 7.2 and from 4.8 to 7.7 for soils RY and RS, respectively (see Fig. 2). The addition of sepiolite resulted in a significant ($p<0.01$) increase in soil pH by about 1.2 and 2.3 pH units on average after the F5 and F10 treatments, respectively. The increase in soil pH after the addition of sepiolite was much larger in soil RS than in soil RY. During the 30-day incubation period, the soil pH firstly declined and then gradually increased. By day 5, soil pH in

Fig. 2 Changes in soil pH with and without sepiolite under different moisture conditions. See Fig. 1 for definitions of NF0, NF5, NF10, F0, F5, F10, RY, and RS. *Bars* indicate the standard deviation of the mean



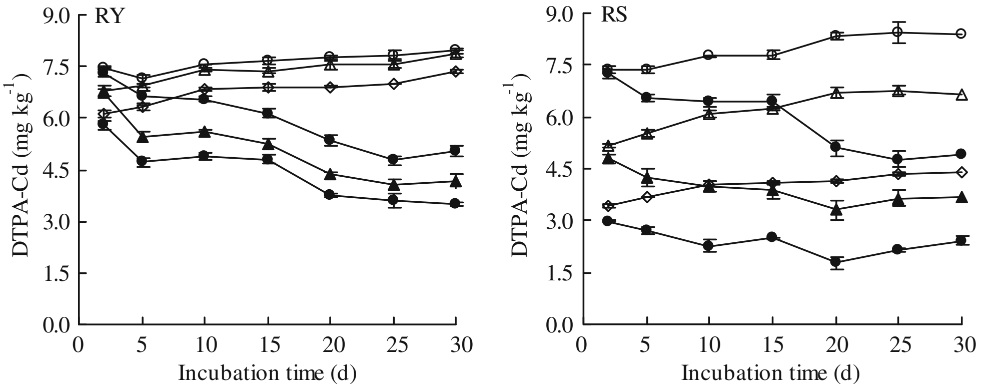


Fig. 3 Changes in DTPA-extractable Cd in soil with and without sepiolite under different moisture conditions. See Fig. 1 for definitions of NF0, NF5, NF10, F0, F5, F10, RY, and RS. *Bars* indicate the standard deviation of the mean

sepiolite-treated soils had decreased by 0.3–0.5 pH units compared with day 2. Following this initial decline, the soil pH gradually increased by 0.7–0.8, 0.4–0.6, and 0.3–0.4 pH units for the F0, F5, and F10 treatments, respectively, between days 5 and 30. Furthermore, the differences in soil pH among the F0, F5, and F10 treatments in soil RY showed a steady decrease throughout the incubation period, whereas they remained generally constant in soil RS.

Relative to the DTPA-extractable Cd levels after the F0 treatment, the F5 and F10 treatments showed a decrease of 0.59–1.15 and 1.15–1.90 mg kg^{-1} in soil RY, and 1.13–2.49 and 2.50–4.29 mg kg^{-1} in soil RS, respectively (see Fig. 3). During the incubation period, the amount of DTPA-extractable Cd in the three treatment groups gradually declined by 31–39% in soil RY, and by 19–33% in soil RS ($p<0.01$). Furthermore, the differences between the amounts of DTPA-extractable Cd after the F0, F5, and F10 treatments in soil RS showed a steady decline throughout the 30-day incubation period. However, no regular change in the response to treatment in soil RY was observed over the incubation period.

Table 2 lists the mean values of soil Eh, pH, and DTPA-extractable Cd in all treatments of both soils on day 30. Following the addition of sepiolite, we observed a significant decrease ($p<0.001$) in soil Eh and DTPA-extractable Cd, and a significant increase ($p<0.001$) in soil pH in soils RY and RS. For both soils, continuous flooding resulted in a significant decrease ($p<0.001$) in soil Eh and DTPA-extractable Cd, and a significant increase ($p<0.001$) in soil pH. Furthermore, we observed significant interactions ($p<0.001$) between sepiolite addition and continuous flooding in terms of soil Eh, pH, and DTPA-extractable Cd in soils.

3.2 Effects of sepiolite and flooding on the fractionation of Cd in paddy soils

Figure 4 shows the proportions of Cd chemical fractions in the two paddy soils before and after incubation. In the

Table 2 Mean values of Eh, pH, and DTPA-extractable Cd in soils treated by sepiolite under both moisture conditions at day 30 ($n=4$ for each treatment)

Treatments	Soil					
	RY			RS		
	Eh (mV)	pH	DTPA-Cd (mg kg^{-1})	Eh (mV)	pH	DTPA-Cd (mg kg^{-1})
NF0	304.5a	4.40f	7.95a	343.3a	5.00e	8.37a
NF5	281.5b	4.99e	7.89a	281.5b	6.60c	6.67b
NF10	252.0c	6.16b	7.33b	239.8c	7.56a	4.38d
F0	−158.0d	5.35d	5.04c	−178.3d	5.62d	4.91c
F5	−181.0e	6.05c	4.18d	−200.5e	6.84b	3.68e
F10	−186.5e	6.81a	3.52e	−205.0e	7.63a	2.42f
Source of variation						
Sepiolite	*	*	*	*	*	*
Flooding	*	*	*	*	*	*
Sepiolite × flooding	*	*	*	*	*	*

Means ($n=4$) followed by the same letter within a row are not significantly different (LSD $p<0.01$)

*$p<0.001$ significance

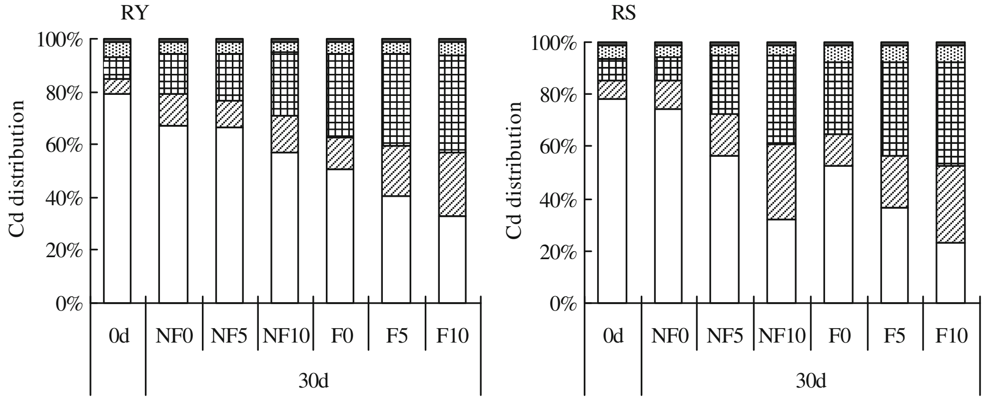


Fig. 4 Changes in Cd fractions in soil with and without sepiolite under different moisture conditions. EX-Cd, exchangeable fraction of Cd in soil (□); CB-Cd, carbonate-bound fraction of Cd in soil (▨); OX-Cd, Fe/Mn oxides-bound fraction of Cd in soil (▦); OC-Cd, organic-matter-bound fraction of Cd in soil (⣿); RES-Cd, residual fraction of Cd in soil (■). See Fig. 1 for definitions of NF0, NF5, NF10, F0, F5, F10, RY, and RS

nonincubated soils (day 0), Cd occurred predominantly in the exchangeable fraction, and this fraction represented about 80% of the total Cd in the two soils. After 30 days incubation, the proportion of EX-Cd relative to total Cd showed a substantial decrease by 3.6–46.1%, and the proportions of CB-Cd and OX-Cd increased by 3.8–21.6% and 1.1–26.0%, respectively, for the two soils compared with nonincubated soils under no flooding condition. Similarly, after 30 days incubation, the proportion of EX-Cd relative to total Cd decreased by 25.4–54.8%, and the proportions of CB-Cd and OX-Cd increased by 4.8–21.6% and 19.4–32.0%, respectively, for the two soils compare with nonincubated soils under continuous flooding condition. Moreover, at the end of the incubation period, EX-Cd had decreased significantly ($p<0.01$) whereas CB-Cd and OX-Cd had increased after the addition of sepiolite (5.0 and 10.0 gkg^{-1}) compared with the levels after no sepiolite addition under both no flooding and continuous flooding conditions. The differences in the proportions of EX-Cd, CB-Cd and OX-Cd between no sepiolite and sepiolite treatments were much larger for continuous flooding soils than for no flooding soils. However, no regular change in OC-Cd or RES-Cd caused by sepiolite treatments under neither no flooding nor continuous flooding conditions was observed.

4 Discussion

For both soils considered in this study, the application of sepiolite and continuous flooding resulted in significant increases ($p<0.01$) in soil pH and decreases in soil Eh (see Figs. 1 and 2; Table 2). Our previous field experiment also yielded an increase in soil pH with the application of sepiolite for remediation of Cd-contaminated paddy soil (soil pH=5.4, Zhu et al. 2010). These changes reflect the high pH of sepiolite (pH=9.3) relative to that of the soils studied. The decrease in soil Eh that accompanies the application of sepiolite may be related to an increase in soil pH (Hu 2006). Previous studies have reported similar changes in soil Eh and pH for soils subjected to continuous flooding (Bjerre and Schierup 1985; Kashem and Singh 2001; Sun et al. 2007). In flooded soil, O_2 is consumed by microbial activity (Eh decrease) and most reduction reactions consume H^+, resulting in an increase in pH in acid soils (Kashem and Singh 2001).

For both of the paddy soils examined in the present study, the application of sepiolite and continuous flooding resulted in a decrease in DTPA-extractable Cd and redistribution of Cd in soils (see Figs. 3 and Fig. 4; Table 2). Furthermore, significantly interaction ($p<0.001$) between sepiolite addition and flooding on immobilization of Cd in both soils were also observed (see Table 2). Previous studies have also reported the immobilization of Cd in soils by sepiolite. For example, Keller et al. (2005) reported that the application of 5% sepiolite markedly reduced DTPA-extractable Cd within the soil in pot experiments and that the application of 1% and 5% sepiolite reduced DTPA-extractable Cd by 13–26% in batch experiments. Álvarez-Ayuso and García-Sánchez (2003) reported similar results in leaching experiments. Our previous field experiment demonstrated that the application of sepiolite to Cd-contaminated paddy soil resulted in a substantial decrease in DTPA-extractable Cd (by 14.5%), and a decrease in EX-Cd, and an increase in CB-Cd, OX-Cd, and RES-Cd; the accumulation of Cd in brown rice decreased by 37.5% as a result of the decreases in DTPA-extractable Cd and in EX-Cd (Zhu et al. 2010). In the present study, the results obtained using a sequential extraction method are in agreement with those obtained using a single extraction (DTPA method). With the application of sepiolite, the concentration of EX-Cd decreased in proportion to the increasing fractions of Cd bound to carbonate or Fe/Mn oxides, under both moisture conditions. A similar redistribution of Cd in the soil has been reported in previous studies of the effects of sepiolite in both pot and field experiments (Xu et al. 2007; Zhu et al. 2010). The findings of these previous studies suggested that the addition of sepiolite to treated soils shifts the solid phases of metals away from their mobile forms to their immobile or less available forms. However, the magnitude of immobilization in our Cd–salt-treated soils was clearly larger than that in the real contaminated soil (as determined in our previous field experiment); this discrepancy may reflect the difference in Cd availability and sepiolite application rate between Cd–salt-treated soils and real Cd-contaminated soils.

Earlier studies have also shown that flooding causes a reduction in the amount of extractable Cd and a redistribution of Cd into different fractions in the soil. For example, Kashem and Singh (2001) reported that during 50 days under flooding conditions, the soluble Cd in the soil decreased by 36–90%. Bjerre and Schierup (1985) found that flooding for 75 days significantly ($p < 0.05$) reduced the $CaCl_2$-extractable Cd in sandy loam soil and sandy soil in pot experiments. In the present study, sequential extraction showed that EX-Cd decreased significantly ($p < 0.01$) with increasing CB-Cd and OX-Cd in the soils. Sun et al. (2007) reported similar changes in the proportions of Cd in the different fractions. These results indicate that flooding can cause the immobilization of Cd in paddy soils. However, few studies have examined the interaction between amendment application and flooding in terms of heavy metal availability. The present results indicate that flooding enhances the stabilization effect of sepiolite on Cd in paddy soils.

Soil pH and Eh are important factors in terms of the availability of heavy metals in the soil (Bjerre and Schierup 1985; Hooda and Alloway 1998). In present study, the amounts of DTPA-extractable Cd and EX-Cd in soil showed significant ($p < 0.01$) positive correlation with Eh and negative correlation with pH, in both the no flooding and continuous flooding soils (Table 3). These results clearly indicate that Cd solubility in the two soils decreased with decreasing Eh and increasing pH. Flooding and the application of sepiolite, as well as the interaction of the two factors, caused the pH to increase and Eh to decrease, which may result in an increased negative charge on the soil particles, and would allow the greater adsorption of metals and consequently a reduction in their mobility (Kashem and Singh 2001; Sun et al. 2007). The affinity of Cd for soil solid phase increases at high pH because Cd tends to form $Cd(OH)^+$ with hydrolysis (Elliott et al. 1986; Sun et al. 2007). Moreover, under the indirect effects of flooding conditions (low Eh), sulfate ions are reduced to the sulfide form, which may form complexes with Cd and immobilize this element as CdS (Van Den Berg et al. 1998; Kashem and Singh 2001). In the present study, these pH- and Eh-dependent mechanisms may have been active after the application of sepiolite to flooded soils, thereby immobilizing Cd. Consistent with the results of the present results, previous studies reported that sepiolite produces new adsorptive surfaces that immobilize Cd in the soil by specific adsorption or chemisorptions; once Cd substitutes for Mg on the edges of the octahedral sheet in sepiolite and is sorbed to the mineral surface, complexation of Cd may occur on surface functional groups (Álvarez-Ayuso and García-Sánchez 2003; Shirvani et al. 2006a, b).

Interestingly, the two tested soils in the present study have similar soil pH, organic matter, and total Cd contents and even distribution of Cd in nonincubated soils; however, the effects of sepiolite application on decrease in DTPA-extractable Cd in soils and a redistribution of Cd in soils were much larger in sandy soil (RS) than in clay soil (RY). Ahmad et al. (2011) also reported that the immobilization effect of alkaline amendments (gypsum and lime) on Cd is greater in sandy loam soil than in sandy clay loam soil. This effect may be attributed to the less buffering capacity of sandy soil than clay soil. In present study, the changes in soil Eh and pH related to sepiolite application and flooding were much larger in sandy soil than in clay soil. These results may indicate that the application of sepiolite as an amendment for Cd is more effective in contaminated sandy soils than in clay soils.

Table 3 Correlation coefficients between the concentration of Cd and soil Eh and pH under different moisture conditions at day 30

Soil	RY				RS			
Moisture condition	NF		F		NF		F	
Extractable Cd	DTPA	EX	DTPA	EX	DTPA	EX	DTPA	EX
Eh	0.885*	0.967*	0.942*	0.900*	0.972*	0.888*	0.868*	0.678**
pH	0.944*	0.988*	0.973*	0.984*	0.973*	0.914*	0.974*	0.841*

NF no flooding, *F* continuous flooding, *DTPA* DTPA-extractable Cd in soil, *EX* exchangeable Cd in soil

*$p < 0.01$ significance; **$p < 0.05$ significance

5 Conclusions

The addition of sepiolite and continuous flooding in typical paddy soils caused a reduction in DTPA-extractable Cd and a redistribution of Cd, with a significant decline in exchangeable Cd and a proportional increase in carbonate-bound and Fe/Mn oxide-bound Cd. Moreover, continuous flooding resulted in an enhanced immobilization effect of Cd in artificially contaminated paddy soil. The effects of sepiolite and continuous flooding on the distribution of Cd are probably indirect, related to their effect on soil Eh (reduction) and soil pH (increase), and to the adsorption capacity of sepiolite itself. The application of sepiolite as an amendment for Cd was much more effective in sandy paddy soil with weaker native sorption capacity than in clay soil. These findings indicate that sepiolite can be used in the remediation of Cd-contaminated paddy soils, especially in sandy paddy soils, and that the water regime plays an important role in stabilizing Cd in paddy soils.

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