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Effect of multi-walled carbon nanotubes on phytotoxicity of sediments contaminated by phenanthrene and cadmium

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HIGHLIGHTS

- Use of MWCNTs for in situ remediation of contaminated sediments was explored.
- Phytotoxicity of contaminated sediments before and after remediation was evaluated.
- Root growth was more sensitive to the changes of pollutant concentration.
- Phytotoxicity might inaccurately indicate the changes of pollutant content.

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ABSTRACT

To implement effective control and abatement programs for contaminants accumulating in sediments, strategies are needed for evaluating the quality of amended sediments. In this study, phytotoxicity of the sediments contaminated by cadmium and phenanthrene was evaluated after in situ remediation with multi-walled carbon nanotubes (MWCNTs) as adsorbents. Adsorption experiments and measurement of aqueous concentrations of the contaminants in overlying water were used to investigate the remediation effectiveness from physical and chemical aspects. The results indicated that MWCNTs showed a much better adsorption performance towards phenanthrene and Cd(II) compared with the sediments. The in situ remediation with MWCNTs could distinctly decrease the aqueous concentrations of phenanthrene and Cd(II) released from the sediments, reducing environmental risk towards overlying water. Influences of MWCNTs dose, MWCNTs diameter, and contact time on phtotoxicity of the contaminated sediments were studied. No significant inhibition of the amended sediments on germination of the test species was observed in the experiments, while the root growth was more sensitive than biomass production to the changes of contaminant concentrations. The analysis of Pearson correlation coefficients between evaluation indicators and associated remediation parameters suggested that phytotoxicity of sediments might inaccurately indicate the changes of pollutant content, but it was significant in reflecting the ecotoxicity of sediments after remediation.

1. Introduction

In situ remediation of contaminated sediments using materials with high adsorption capacity has become a common method due to low cost, simple operation, and less impact on natural hydrological conditions (Peng et al., 2009; Gomes et al., 2013; Zhang et al., 2016). This remediation technique aims at improving the stabilization of pollutants in sediments by reducing their mobility, bioavailability, and toxicity with adsorbents. For this purpose, many nanomaterials (e.g., carbon nanotubes, nano-hydroxyapatite, nano-TiO_2, and nanoscale zero-valent iron) with stronger affinity for pollutants than traditional materials (e.g., activated carbon, biochar, and zeolite) are explored for in situ remediation of contaminated sediments (Ferguson et al., 2008; Tang et al., 2008; Feng et al., 2010; Zhang et al., 2010; Xu et al., 2012a; Tomašević et al., 2014; Zhang

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et al., 2014).

Carbon nanotubes, including single-walled carbon nanotubes and multi-walled carbon nanotubes (MWCNTs), are regarded as one of the most promising nanomaterials as adsorbents for removing pollutants (Gong et al., 2009). Recently, several studies have investigated the remediation of contaminated sediments with carbon nanotubes (Kwadijk et al., 2013; Abbasian et al., 2016; Tričković et al., 2016). The results of these studies provide evidence that carbon nanotubes can effectively decrease ecological risk of contaminated sediments. However, stabilized contaminants still remain in sediments. When the hydrological conditions change, contaminants may be released into overlying water again (Zeng et al., 2013). Therefore, it is necessary to evaluate the effectiveness of in situ remediation of contaminated sediments. Additionally, in situ remediation is a site-specific process, full-scale field applications of the remediation also calls for effective evaluation methods (Apitz et al., 2004).

Most of current evaluations focus on the total amount of contaminants. Nevertheless, it is difficult to determine the immobilized contaminants directly. Some researchers used polyethylene passive samplers (Choi et al., 2014; Pisanello et al., 2016) or Rhizon soil moisture samplers (Park et al., 2011; Sumon et al., 2012) to measure the decreased amount of contaminants in pore water. These methods are valuable for characterizing the remediation effect, but they do not illuminate the significant processes that affect the bioavailability of contaminants. Moreover, they are expensive and time-consuming. Phytotoxicity is a significant part of the ecological risk assessment of contaminants. Compared with the toxicity tests using animals, algae, and microorganisms, phytotoxicity tests during early germination have certain advantages. (1) Dry plant seeds exhibit a better applicability under harsh and rapidly changing environment. (2) Plant seeds are much cheaper and can be stored for a longer time. (3) The tests can be simple, sensitive, and fast. (4) There is no need to add plant nutrients during the tests (Wang et al., 2001; Huang et al., 2008; Kwon et al., 2016; Song et al., 2016). Thus, phytotoxicity of the sediment during early germination can be a convenient, fast, and effective way to reflect the quality of sediment.

Polycyclic aromatic hydrocarbons (PAHs) and heavy metals (HMs) are two widespread pollutants in sediment. Their fates are of great environmental concern due to their toxic, mutagenic, and carcinogenic properties (Hong et al., 2016; Sá et al., 2016; White et al., 2016). In this study, cadmium and phenanthrene are selected as model compounds of HMs and PAHs. The objectives of the study were (1) to carry out in situ remediation of sediments contaminated by phenanthrene and cadmium with MWCNTs, and (2) to assess phytotoxicity of the contaminated sediments with and without addition of MWCNTs by selected plants with short germination period as bio-indicators.

2. Materials and methods

2.1. Chemicals, carbon nanotubes and sediments

Phenanthrene ($C_{14}H_{10}$, purity > 97%) was purchased from Xiya Chemical Industry Co., Ltd., Shandong, China. Cadmium chloride ($CdCl_2 \cdot 2.5H_2O$, analytical grade) was obtained from Sinopharm Chemical Reagent Co., Ltd., Shanghai, China. Three MWCNTs (purity > 95%) with different outer diameter (10–20 nm, 30–50 nm, >50 nm) were used in this study. They were purchased from Chengdu Organic Chemistry Co., Chinese Academy of Sciences, Chengdu, China.

Surface sediment samples (0–15 cm) were collected from the Xiangjiang River in Changsha, Hunan Province, China. All sediment samples were air-dried at room temperature and crushed in a porcelain mortar. Then the samples were sieved over a 2 mm mesh sieve and homogenized prior to use. Sediment properties including pH, cation exchange capacity, organic carbon content, zeta potential, electrical conductivity, and texture were measured according to previously reported methods (Bouyoucos, 1928; Gillman and Sumpter, 1986; Yeomans and Bremner, 1988; Su et al., 2016).

2.2. Adsorption experiments

Adsorption of the two contaminants on sediments and MWCNTs was conducted in 50 mL glass bottles with Teflon-lined screw-cap. For the adsorption of phenanthrene, 2 g L^{-1} sediments or 0.1 g L^{-1} MWCNTs were mixed with phenanthrene solutions of various concentrations (0.1–4 mg L^{-1}) on a shaker for 48 h at 180 rpm, 25 ± 1 °C. After equilibrium, the suspension was centrifuged and the phenanthrene concentration in supernatant was determined by high performance liquid chromatography (HPLC, Agilent 1100, USA). Initial phenanthrene solutions of various concentrations were prepared by diluting concentrated phenanthrene stock solutions using methanol as solvents. The final concentration of methanol in the aqueous solution was kept below 0.1% (v/v) to minimize the cosolvent effect (Nkedi-Kizza et al., 1985; Chen et al., 2007). For the adsorption of Cd(II), the experiments were carried out by mixing 0.5 g L^{-1} sediments or 0.5 g L^{-1} MWCNTs with Cd(II) solutions of varying initial concentrations (2–20 mg L^{-1}) on a shaker for 12 h at 180 rpm, 25 ± 1 °C. After equilibrium, the supernatants were taken out and filtered through filter membranes with 0.45 μm pore size. The Cd(II) concentration in the filtrate was measured by an atomic absorption spectrometer (AAS, Agilent 3510, USA).

2.3. Sediment-spiking procedures

Quantitative phenanthrane or cadmium chloride was spiked into the sediments to reach desired concentrations according to previously reported methods with appropriate modifications (Brinch et al., 2002; Simpson et al., 2004; Jiang et al., 2015). For thorough mixing of phenanthrane and sediments, penanthrane was dissolved in dichloromethane and then added to 25% of the total weight of dry sediments, followed by stirring every 15 min to evaporate the solvent completely. Subsequently, the treated sediments were mixed with the rest 75% of sediments. After manual homogenisation, the moisture content of spiked-sediment was adjusted by adding 50% (v/w) ultrapure water. Following that, the blending container was sealed and stored in darkness to avoid evaporation and photolysis of phenanthrene. The Cd(II) was artificially added into sediments by adding contamination solutions using deoxygenated water as homogenising solvent. Spiked sediments were homogenized using a glass stirring rod. After that, the mixture was deoxygenated by bubbling with nitrogen for 2 h to minimize oxidation reactions that cause the pH value to decrease (Simpson et al., 2004). The spiked sediments were shaked periodically and aged for 6 weeks to achieve equilibrium of diffusion processes within the sediments (Oliver, 1987). Samples were taken out to determine final concentrations of Cd(II) and phenanthrene in the sediment after spiking procedure. According to the consequences of measurement, sediments with actual concentration of 1.42 mg Cd(II) or 2.56 mg phenanthrene per gram of dry weight sediment were used for the following experiments.

2.4. Amendment of contaminated sediments

To determine the effect of MWCNTs dose on remediation effectiveness of the contaminated sediments, MWCNTs were mixed with the sediments at the dose of 0.5%, 1.0%, or 1.5% (w/w). In the experiment exploring the influence of different particle size of MWCNTs on remediation effectiveness, three MWCNTs with different outer diameter (10–20 nm, 30–50 nm, >50 nm) were used. The contaminated sediments were amended with these MWCNTs at the dose of 1.5% (w/w). To obtain further insight into the remediation process, sediments were mixed with MWCNTs (10–20 nm) at the dose of 1.5% (w/w) and shaked periodically in darkness for 7, 30, and 60 days to investigate the effect of contact time on remediation effectiveness.

2.5. Aqueous concentrations of contaminants released from sediments

Glass bottles containing 10 g treated or untreated sediments were filled with 50 mL ultrapure water and 1 g L^{-1} sodium azide. These bottles were shaken at 30 rpm for 48 h. Subsequently, the mixture of sediment and water was allowed to static settlement for 10 days. After that, triplicate water samples were taken out, following by the analytical procedure of HPLC and AAS with the methods described above.

2.6. Phytotoxicity tests during early germination

The phytotoxicity tests with *Phaseolus radiatus* L. (mung bean) and *Raphanus sativus* L. (radish) were conducted according to the OECD Guidelines for the Testing of Chemicals with some modifications (OECD, 2006). Before sowing, the seeds were sterilized with 10% H_2O_2 for 10 min to prevent fungal growth and stimulate germination, and then rinsed copiously with deionized water. After that, ten seeds were sown on sediment or amended sediment in Petri dishes. The moisture content was maintained at about 60% of the water holding capacity of the sediment and adjusted daily. The Petri dishes were placed in a thermostatic incubator at 25 °C with light conditions of 14 h of daylight and 10 h of darkness per day. The experiments were terminated after 72 h cultivation. Seed germination rate, length of the root and weight of fresh biomass were measured. Each treatment was replicated four times.

2.7. Data analysis

Data were graphed using Origin 8.0 software (OriginLab Corporation, Massachusetts, USA). Microsoft Excel 2013 (Microsoft Corporation, Washington, USA) and SPSS Statistics 22 (IBM Corporation, New York, USA) were used for numerical data processing. Analysis of the variance (ANOVA) was performed to test differences between each two compared groups. The Pearson correlation analysis was carried out to test the significance of correlation between each two variables.

3. Results and discussion

3.1. Characterization of the sediments and MWCNTs

The pH value of the sediment was 7.94, the cation exchange capacity was 10.89 cmol kg^{-1}, the organic carbon content was 1.58%, and it had a composition of 47.60% clay, 28.87% silt, and 23.53% sand. The zeta potential and electrical conductivity of the sediment were −18.4 mV and 0.145 mS cm^{-1}, respectively. MWCNTs used in the experiments were characterized by scanning electron microscope (SEM), Fourier translation infrared spectrum (FT-IR), and BET surface analyzer to determine the situation of the surface of nanotube. The characterization results are presented in Fig. 1. In the infrared spectrum, the bands at about 3500 cm^{-1} are attributed to the presence of hydroxyl groups (—OH), which may result from the oxidation of MWCNTs during the purification or the atmospheric water bound to the MWCNTs. And the bands at about 1600 cm^{-1} are believed to be caused by C=O stretching of quinone groups on the surface of the MWCNTs (Ma et al., 2006). Besides, the

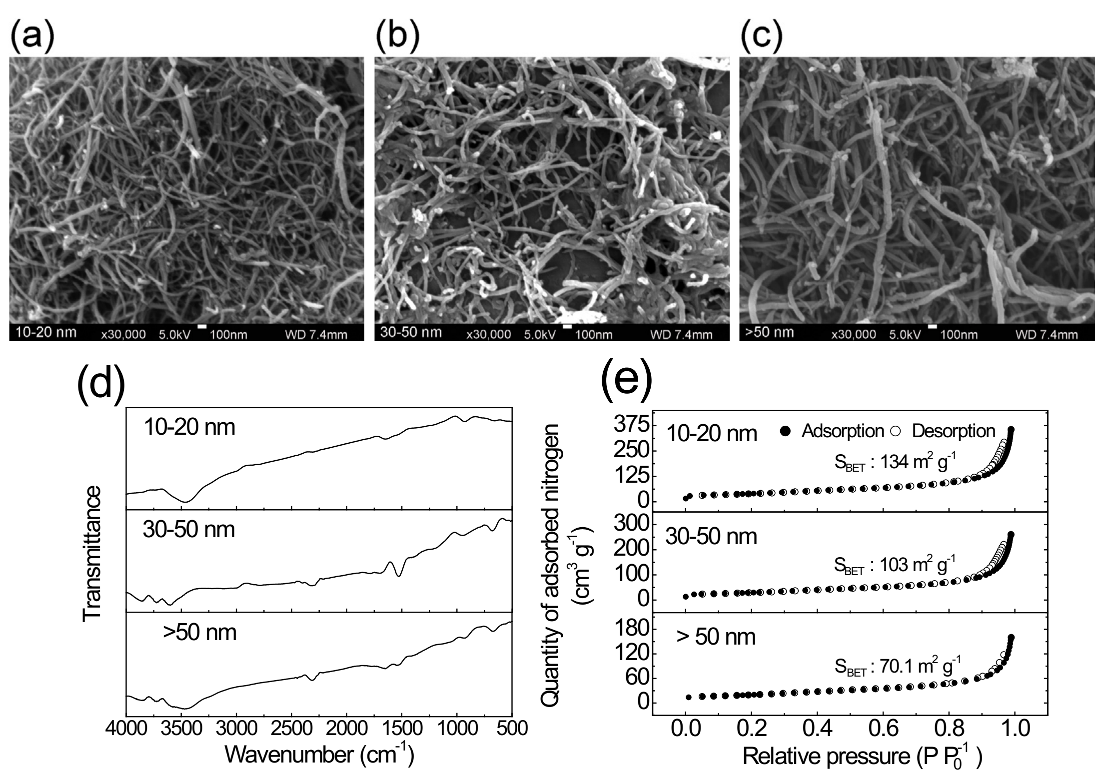

Fig. 1. SEM images (a, b, and c), FT-IR spectrum (d), and nitrogen adsorption-desorption isotherms for BET surface analysis (e) of the used MWCNTs.

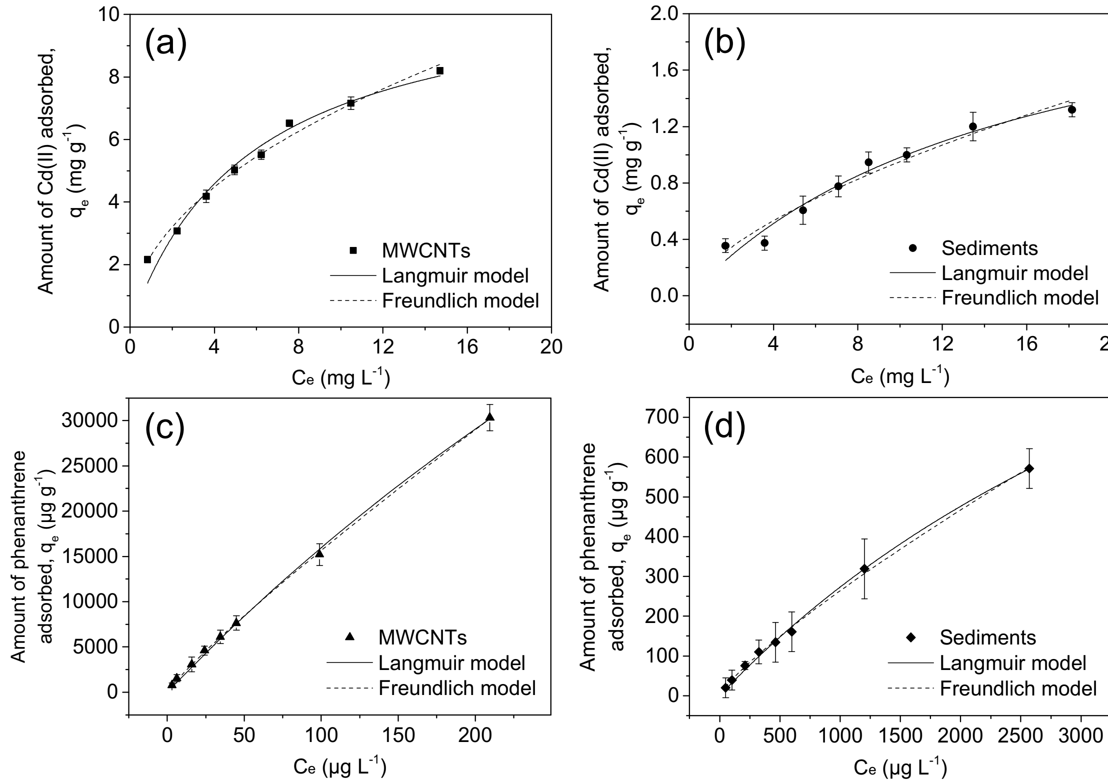

Fig. 2. Measured (dots) and model fitted (lines) isotherms of Cd(II) adsorption by MWCNTs (a) and sediments (b), and phenanthrene adsorption by MWCNTs (c) and sediments (d).

measured BET specific surface area of 10–20 nm, 30–50 nm, and >50 nm MWCNTs are 134 $m^2\ g^{-1}$, 103 $m^2\ g^{-1}$, and 70.1 $m^2\ g^{-1}$, respectively.

3.2. Adsorption of the contaminants on sediments and MWCNTs

The adsorption isotherms of Cd(II) and phenanthrene are shown in Fig. 2. Langmuir and Freundlich models (Fan et al., 2008) are used to describe the adsorption performance of sediments and MWCNTs for the two contaminants. The models are given by the following equations:

$$\text{Langmuir model}: \ q_e = \frac{q_m K_L C_e}{1 + K_L C_e} \quad (1)$$

$$R_L = \frac{1}{1 + K_L C_0} \quad (2)$$

$$\text{Freundlich model}: \ q_e = K_F C_e^{\frac{1}{n}} \quad (3)$$

where q_e (mg g^{-1}) is the amount of adsorbed Cd(II) or phenanthrene at equilibrium, q_m (mg g^{-1}) is the maximum adsorption capacity, K_L (L mg^{-1}) is the Langmuir constant related to the bond energy of the adsorption reaction between adsorbate and adsorbent, C_e (mg L^{-1}) is the equilibrium concentration of Cd(II) or phenanthrene. R_L (a dimensionless constant) is the equilibrium parameter to predict the type of isotherm: unfavorable equilibrium ($R_L > 1$), linear case ($R_L = 1$), favorable equilibrium ($0 < R_L < 1$), or irreversible case ($R_L = 0$) (Hall et al., 1966), C_0 (mg L^{-1}) is the initial concentration of Cd(II) or phenanthrene. K_F [mg g^{-1}(mg L^{-1}) $^{-1/n}$] represents the quantity of adsorbate adsorbed onto adsorbent for a unit equilibrium concentration, n (dimensionless) is an indicator of adsorption intensity.

Fitting parameters of the isotherm models are presented in Table 1. It was observed that the adsorption data were well fitted by Freundlich and Langmuir models with all the values of $R^2 \geq 0.9622$. According to the calculated values of K_F and q_m, the adsorption capacity of phenanthrene on MWCNTs was much higher than that of Cd(II) on MWCNTs. It could be due to the strong π-π electron-donor-acceptor interaction between phenanthrene molecules and the highly polarizable graphene sheets of MWCNTs (Chen et al., 2007). All the R_L values were between zero and one, which indicated that the adsorption of Cd(II) and phenanthrene on the adsorbents was favorable (Hall et al., 1966; Hu et al., 2011). Furthermore, the R_L values decreased with the increase of initial

Table 1
Parameters of the isotherm models for Cd(II) and phenanthrene adsorption.

Adsorbate	Adsorbent	Langmuir				Freundlich		
		K_L (L mg^{-1})	q_m (mg g^{-1})	R_L	R^2	K_F (mg g^{-1} (mg $L^{-1})^{-1/n}$)	$1/n$	R^2
Cd(II)	MWCNTs	0.1737	11.18	0.2235–0.7422	0.9743	2.292	0.4833	0.9887
Cd(II)	Sediments	0.06391	2.518	0.4389–0.8867	0.9694	0.2213	0.6337	0.9622
Phenanthrene	MWCNTs	1.092	162.1	0.1863–0.9016	0.9986	121.4	0.8895	0.9997
Phenanthrene	Sediments	0.1726	1.856	0.5916–0.9830	0.9979	0.2638	0.8238	0.9984

adsorbate concentration, demonstrating that the adsorption process was more favorable with higher initial concentration of Cd(II) or phenanthrene. A value of $1/n$ below one indicates a normal Freundlich isotherm, while $1/n$ above one implies cooperative adsorption (Dada, 2012). And the adsorption becomes more heterogeneous as $1/n$ gets closer to zero (Xu et al., 2012b). In our study, all the values of $1/n$ were below one (Table 1), indicating that the Freundlich isotherms were normal. The adsorption process of Cd(II) was more heterogeneous than that of phenanthrene with lower values of $1/n$ (0.4833 and 0.6337). Based on the results, the obtained MWCNTs showed a much better adsorption performance towards phenanthrene and Cd(II) compared with the sediments. And the consequences of adsorption isotherms provided useful information for the in situ remediation of sediments contaminated by phenanthrene and Cd(II) with MWCNTs.

3.3. Aqueous concentrations of contaminants in overlying water

Under natural conditions, contaminants in sediment are likely to diffuse from sediment into overlying water through the sediment-water interface. It is of great importance to measure the contaminant concentration in the overlying water when investigating the influence of contaminated sediment on overlying water and assessing the effectiveness after remediation (Apitz et al., 2004, 2005). Results of the measurement of phenanthrene and Cd(II) concentrations in overlying water are shown in Fig. 3. It was proved that the in situ remediation with MWCNTs could distinctly reduce the concentrations of phenanthrene and Cd(II) released from the sediments. And the reduction of phenanthrene concentrations was more obvious. The results could be explained by the higher adsorption capacity of MWCNTs towards phenanthrene according to the adsorption experiments. The concentration of Cd(II) decreased with the increasing dose of MWCNTs, however, there were no significant differences ($P > 0.05$) between groups of phenanthrene when amended with various dose of MWCNTs. Besides, the reduction of phenanthrene concentrations decreased when MWCNTs with larger diameter were used, while no significant differences were observed in the experimental groups of Cd(II). It could also be found that the aqueous Cd(II) concentrations reduced with the prolonged period of contact time, but no obvious change of aqueous phenanthrene concentrations was observed after 60 days. As representative chemical indicators, the aqueous concentrations of contaminants released from sediments are relatively accurate and convincing. Hence, they can be utilized to assess the effectiveness of remediation and act as references when investigating the phytotoxicity of amended sediments.

3.4. Phytotoxicity of amended sediments

For the contaminated sediments and the tested seeds, no significant inhibition of germination (fluctuation of germination rates was less than 5%) was observed in the experiments. On this account, the inhibition of root growth and biomass (roots and shoots) production were chosen for phytotoxicity evaluation during the early germination of the seeds. The percentages of root growth inhibition (RGI) and biomass production inhibition (BPI) were calculated with the following formula:

$$\text{RGI or BPI }(\%) = (\text{A} - \text{B})/\text{A} \times 100 \quad (4)$$

where A denotes the mean root length (mm) or fresh biomass (g) in the control (uncontaminated sediment), B is the mean root length (mm) or fresh biomass (g) in the test.

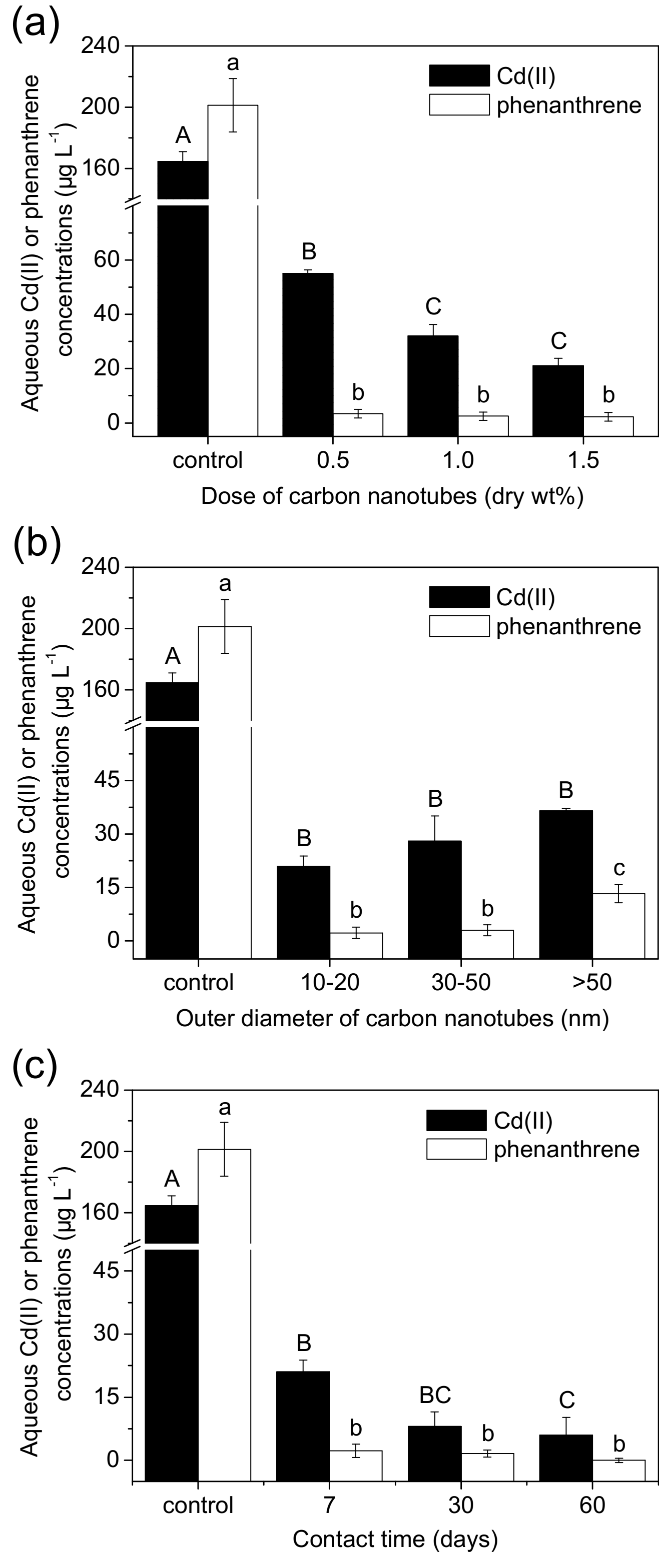

Fig. 3. Aqueous concentrations of Cd(II) and phenanthrene released from sediments under different remediation conditions: various dose (a) and outer diameter (b) of MWCNTs, different contact time (c) between sediments and MWCNTs. The control groups were untreated with MWCNTs. Different letters denote statistically significant differences ($P \leq 0.05$) between bars.

3.4.1. Phytotoxicity of phenanthrene and Cd(II)

Both phenanthrene and Cd(II) exhibited phytotoxicity towards *P. radiatus* and *R. sativus*. And the root elongation and fresh biomass

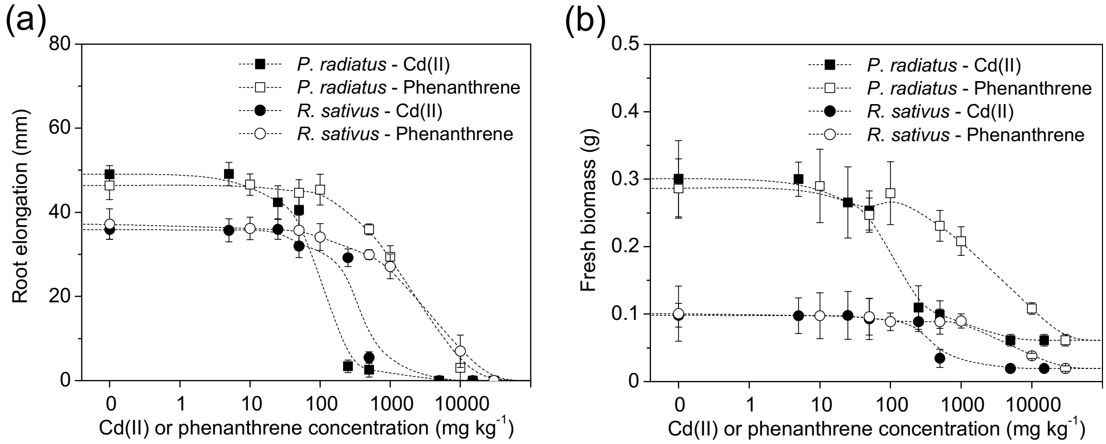

Fig. 4. Changes of root elongation (a) and fresh biomass (b) as a function of the Cd(II) or phenanthrene concentration in sediments. Error bars represent standard deviations ($n = 4$).

varied with the change of phenanthrene and Cd(II) concentration in the sediments (Fig. 4). It was observed that the value of root elongation started to decline at a relatively lower concentration of Cd(II) than that of phenanthrene (Fig. 4a) and the fresh biomass reduced more rapidly when they were exposed to Cd(II) (Fig. 4b). These results indicated that the tested seeds were more sensitive to Cd(II) than phenanthrene. The estimated 50% effective concentration (EC_{50}) of root growth inhibition were 96.5 mg kg^{-1} (*P. radiates*) and 332.3 mg kg^{-1} (*R. sativus*) for Cd(II), 1.65 g kg^{-1} (*P. radiates*) and 2.66 g kg^{-1} (*R. sativus*) for phenanthrene, respectively. Such results suggested that the roots of *R. sativus* were more tolerant to Cd(II) and phenanthrene in sediments than the roots of *P. radiates* when considering the species differences. Different alteration of toxicity symptoms might result from different toxic mechanisms of cadmium and phenanthrene. Cadmium is a non-essential element for plant growth. Due to its high water solubility and mobility, cadmium is easy to enter the roots. By binding with biomacromolecule (e.g., proteins, organic acids, and phytochelatins) and stimulating the formation of free radicals, the toxic metal can cause disruption of structure and inhibition of physiological activity (Gallego et al., 2012; Andresen et al., 2016; Rizwan et al., 2016). Phenanthrene can also induce alteration of enzyme activities and oxidative stress (Wei et al., 2014). Besides, owing to its hydrophobic property, phenanthrene may inhibit or reduce water and gas exchange as well as the nutrients absorption of seedlings (Khan et al., 2013, 2014; Cheng et al., 2016).

3.4.2. Effect of MWCNTs dose

The phytotoxicity (inhibition of root growth and biomass production) of the amended sediments depended on the MWCNTs dose (Fig. 5a and b). Uncontaminated sediments of control groups contained MWCNTs at corresponding concentrations (0.5%, 1.0%, and 1.5%; w/w), and no significant inhibition of root growth and fresh biomass production was observed in the control groups (data not shown). For the sediments contaminated by Cd(II), the root growth inhibition of *P. radiates* and *R. sativus* was reduced with the increasing dose of MWCNTs. Though MWCNTs caused reduction of biomass production inhibition of *R. sativus*, there were no distinct differences between the experimental groups. For *P. radiates*, only the group containing MWCNTs at the dose of 1.5% showed a significant difference in the inhibition of biomass production compared with the control group. As to the sediments contaminated by phenanthrene, MWCNTs obviously reduced the phytotoxicity. A higher dose of MWCNTs reduced the inhibition of root growth to a greater degree. However, no significant differences were observed in the inhibition of biomass production, except for *R. sativus* after the application of MWCNTs at the dose of 1.5%. Moreover, negative values of the inhibition of root growth (−3.6%) and biomass production (−5.29%) were observed, which means that the MWCNTs (1.5%) amended sediments showed a positive effect on the root growth and biomass production of *R. sativus*. The reason is likely that MWCNTs improved the adsorption of water and essential nutrients, promoted cell elongation in the root system, and increased dehydrogenase activity, resulting in faster root growth and higher biomass production (Wang et al., 2012; Tiwari et al., 2013).

Based on the results above, the application of MWCNTs in the sediments not only reduced the concentrations of contaminants, but also diminished the phytotoxicity. In a previous study (Jośko et al., 2013), the increase of MWCNTs dose in the sediments caused a lower reduction of phytotoxicity. The result may be attributable to the use of greater MWCNTs dose. Their sediments were mixed with adsorbents at the dose of 1, 5, or 10% (w/w), much more than this study. Thus, it cannot be ignored the negative effect of MWCNTs, such as the clogging of pores, the inhibition of transport of nutrients and water, and the accumulation of partial contaminants (Jośko et al., 2013). In addition, it was found that the root growth was more sensitive than biomass production to the change of contaminant concentrations as roots were directly exposed to the contaminants in sediments. Besides, the reduction of root tissue mass under stress of contaminants might also decrease the growth regulators transported from root to shoot, affecting shoot growth and biomass production (Bernstein et al., 2004).

3.4.3. Effect of MWCNTs outer diameter

As the root growth was a more sensitive parameter, the following studies mainly focused on the inhibition of root growth. The effect of outer diameter of MWCNTs on phytotoxicity was displayed in Fig. 5 c and d. Application of MWCNTs effectively reduced the root growth inhibition of both *P. radiates* and *R. sativus*. But for Cd(II) (Fig. 5c), no significant differences were found between the experimental groups of *P. radiates*, yet the MWCNTs with a smaller diameter (10–20 nm) showed greater reduction of root growth inhibition of *R. sativus*. The results indicated that the root of *R. sativus* was more sensitive than that of *P. radiates* in response to small changes at the tested concentrations. As for sediments contaminated by phenanthrene (Fig. 5d), the group using MWCNTs with the largest diameter (>50 nm) still reduced the inhibition of root growth to a certain extent, although not as much as the other two groups.

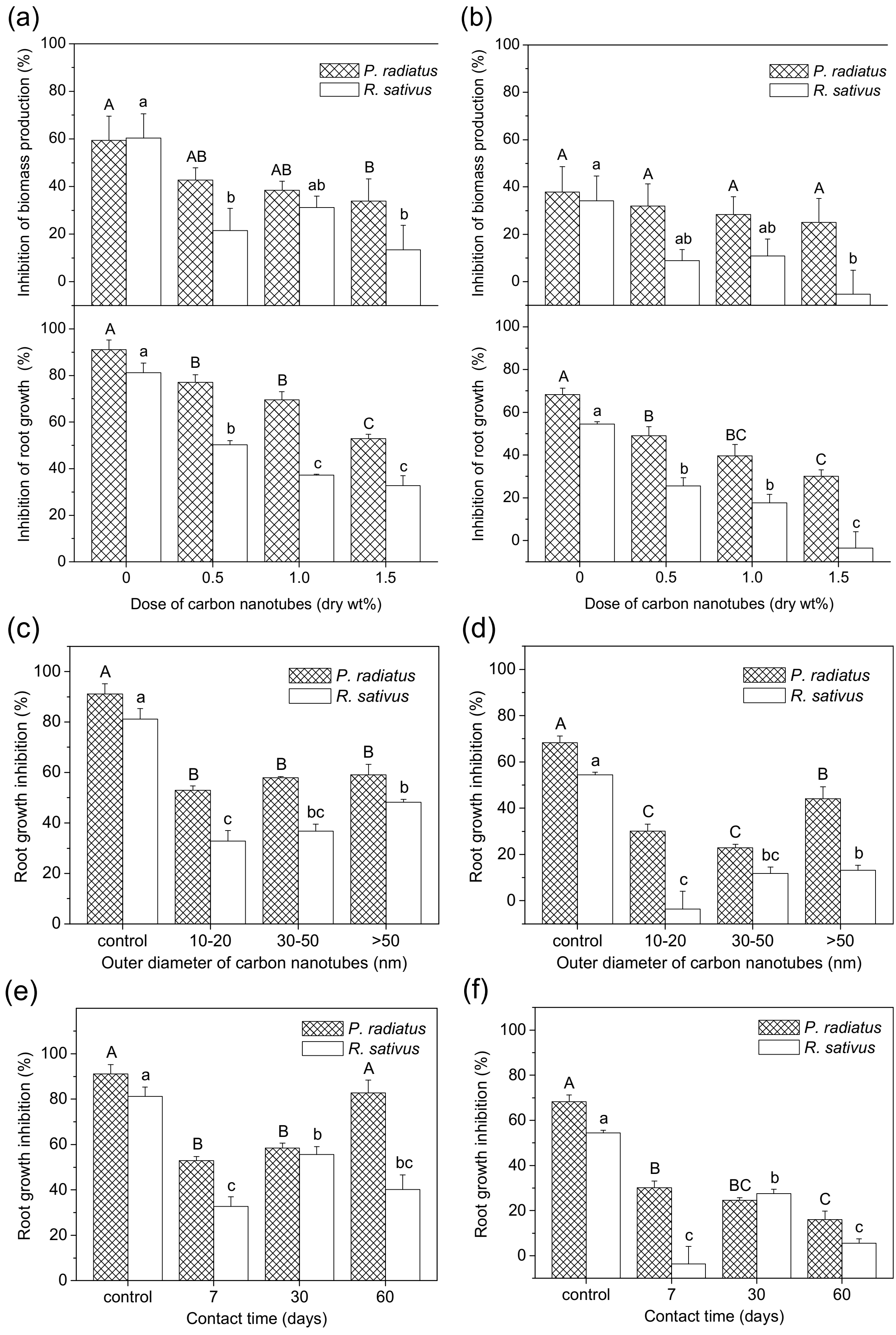

Fig. 5. Effect of MWCNTs dose, outer diameter of MWCNTs, and contact time on phytotoxicity (inhibition of root growth and biomass production) of sediments contaminated by Cd(II) (a, c, e) and phenanthrene (b, d, f). Error bars represent standard deviations ($n = 4$). Different letters denote statistically significant differences ($P \leq 0.05$) between bars.

Table 2
Pearson correlation coefficients (r) between evaluation indicators and different parameters associated with the remediation.

Parameters	Cd (II)			Phenanthrene		
	Aqueous concentration	Phytotoxicity (root growth inhibition)		Aqueous concentration	Phytotoxicity (root growth inhibition)	
		P. radiates	*R. sativus*		*P. radiatus*	*R. sativus*
MWCNTs dose	−0.980	−0.977	−0.243	−0.946	−0.999	−0.968
MWCNTs outer diameter	0.999	0.936	0.968	0.901	0.663	0.895
Contact time	−0.889	0.963	0.248	−0.993	−0.998	0.211

According to the measurement of BET specific surface area, 10–20 nm MWCNTs exhibit higher specific surface area (134 $m^2\ g^{-1}$) than 30–50 nm MWCNTs (103 $m^2\ g^{-1}$) and >50 nm MWCNTs (70.1 $m^2\ g^{-1}$). A higher specific surface area offers more sites for adsorption or interaction (Xu et al., 2012b), enabling 10–20 nm MWCNTs to be more effective in stabilizing pollutants. Additionally, the size of MWCNTs may cause different changes in sediment structure, further influencing the availability of water and nutrients during the root growth process (Jośko et al., 2013; Mayer et al., 2014). And this could also be the reason why the results of phytotoxicity were not always in keeping with the consequences of aqueous concentration in overlying water.

3.4.4. Effect of contact time

The contact time clearly influenced the effectiveness of MWCNTs in reduction of phytotoxicity of sediments (Fig. 5e and f). In field applications, long-term effectiveness of the remediation method is key to ensuring successful implementation. For *P. radiates*, it was found from Fig. 5e that the reduction of root growth inhibition was at a similar level after 7 and 30 days of contact, but decreased after 60 days. And for *R. sativus*, a less reduction of phytotoxicity was observed after 30 days of contact. In the case of sediments contaminated by phenanthrene (Fig. 5f), for *P. radiates*, the reduction of toxicity was increased with the prolonged contact time. However, the value of root growth inhibition of *R. sativus* was significantly increased after 30 days.

Previous studies also reported the negative effect of contact time on reduction of the phytotoxicity (Oleszczuk et al., 2012; Jośko et al., 2013). They explained that the reason behind this could be the reduction of the availability of nutrients and water due to their immobilization by the MWCNTs. Conversely, Maila et al. (Maila and Cloete, 2002) observed a positive effect of contact time on reducing the phytotoxicity due to the dissipation of PAHs in the soil, which was in accord with the consequences of aqueous concentrations in overlying water. Therefore, the contaminants in the sediment were no longer the dominant factors affecting seedlings growth after long time contact. Physical and chemical properties of the sediments probably changed during the longtime aging. The unfavorable factors (such as mechanical impedance, poor aeration and water availability, deficiencies of the essential nutrient elements, and unsuited pH value) of the sediments could also restrict the root growth of seedlings (Eavis, 1972; Foy, 1992).

3.5. Relationships between phytotoxicity and associated parameters

Pearson correlation coefficient (r) is commonly used as a statistical metric by experimental scientists to measure the correlation relationship between two random variables (Wang et al., 2015). In this study, validity of the evaluation methods is expressed by the Pearson correlation coefficients between evaluation indicators and different parameters associated with the remediation. Calculated coefficients were displayed in Table 2. For accurate calculation, Pearson correlation coefficients between MWCNTs outer diameter and evaluation indicators were calculated using specific surface area to replace the imprecise outer diameter. A relatively stronger correlation was observed between aqueous concentrations of pollutants in overlying water and tested remediation parameters. However, the relationships between the phytotoxicity and tested remediation parameters varied a lot. Such results indicated that the aqueous concentrations of pollutants were more stable and reliable than phytotoxicity indicators in reflecting the changes of pollutant amount. The Pearson correlation coefficients in the group of *P. radiatus* were mostly higher than that in the group of *R. sativus*, which suggested that *P. radiatus* was a more stable indicator. Besides, it is worth noting that negative correlations ($r = -0.889$ and $r = -0.993$) between contact time and aqueous concentrations were found, but data also showed positive correlations ($r = 0.963$, $r = 0.248$, and $r = 0.211$) between contact time and phytotoxicity. The opposite results involved two kinds of implications. On the one hand, phytotoxicity of sediments might inaccurately indicate the changes of pollutant content. On the other hand, in situ treatment with MWCNTs probably increased the ecotoxicity of sediments after a long time remediation.

4. Conclusions

In this work, phytotoxicity of sediments contaminated by phenanthrene and cadmium was determined following in situ remediation with MWCNTs. The remediation treatment could not only decrease the aqueous concentrations of phenanthrene and Cd(II) released from the sediments, reducing the environmental risk towards overlying water, but also diminish the phytotoxicity of the contaminated sediments. No significant inhibition of the amended sediments on germination was observed in the experiments, while the root growth was more sensitive than biomass production to the changes of contaminant concentrations. Prolonging the period of contact time decreased the reduction of phtotoxicity of the contaminated sediments. Based on the results of this study, using phytotoxicity of sediments for remediation assessment can be simple and quick, and it is significant in reflecting the ecotoxicity of sediments after remediation. However, the phytotoxicity might inaccurately indicate the changes of pollutant content, and more plant species need to be studied in future research.

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