Influence of the phenols on the biogeochemical behavior of cadmium in the mangrove sediment

Jian Li [a,c], Jingchun Liu [a], Haoliang Lu [a], Hui Jia [a], Junyi Yu [a], Hualong Hong [a], Chongling Yan [a,b,*]

[a] Key Laboratory of Ministry of Education for Coastal and Wetland Ecosystems, Xiamen University, Xiamen, 361102, China
[b] State Key Lab of Marine Evironmental Science, Xiamen University, Xiamen, 361102, China
[c] Institute of Environment and Ecology, School of the Environment and Safety Engineering, Jiangsu University, Zhenjiang, China

HIGHLIGHTS

- Phenols disturbed the "source-sink" balance of Cd and turned it into a "source".
- Phenols played a protective role in the plants of contaminated mangroves.
- Phenols facilitated Fe absorption and alleviated the limit of Fe to the plant growth.

ARTICLE INFO

ABSTRACT

Phenols exert a great influence on the dynamic process of Cd in the soil-plant interface. We investigated the influence of phenols on the biogeochemical behavior of cadmium in the rhizosphere of *Avicennia marina* (Forsk) Vierh. All combinations of four levels of cadmium (0, 1, 2 and 4 mg/kg DW) and two levels of phenol (0 and 15 mg/kg DW) were included in the experimental design. We found that phenols facilitated increasing concentrations of exchangeable cadmium (Ex-Cd), acid volatile sulfide (AVS) and reactive solid-phase Fe (II) in sediments, and iron in plants, but inhibited Cd accumulation in iron plaque and roots. The concentrations of AVS and reactive solid-phase Fe (II) were significantly positively correlated with Cd treatment. As for the biogeochemical behavior of Cd in mangrove sediments, this research revealed that phenols facilitated activation and mobility of Cd. They disturbed the "source-sink" balance of Cd and turned it into a "source", whilst decreasing Cd absorption in *A. marina*. Additionally, phenols facilitated iron absorption in the plant and alleviated the Fe limit for mangrove plant growth.

© 2015 Elsevier Ltd. All rights reserved.

1. Introduction

Mangrove forests are located in the intertidal zone of tropical and subtropical regions as a specific ecological habitat, and play a key role in maintaining the coastal ecological balance (Liu et al., 2010; Du et al., 2013). Mangrove sediments are known as the final "sink" for discharge of heavy metals into the seawater (Tam and Wong, 1997; Chou et al., 2004). However mangrove ecosystems are subjected to serious heavy metal pollution, such as Cd, Cu, Pb and Cr (MacFarlane et al., 2003; Silva et al., 2006). *Avicennia marina*, the pioneer mangrove species in the southeast of China, has been found to accumulate heavy metals in root tissues under field conditions with levels equal to or greater than surrounding sediment concentrations (MacFarlane et al., 2003). One of the heavy metals, Cd, has drawn more attention because of its high bioavailability and activity, which interferes with plant metabolic processes, leading to poor growth and lower biomass.

Soils developed from peat have an abundance of phenolic compounds and other organic matter which can adsorb a large quantity of heavy metals. Our preliminary studies have revealed a high content of phenols in mangrove sediments, from about 20 mg/kg DW to 28 mg/kg DW (Table 1). The sources of phenols are mainly root exudates, secretions of microorganisms and release by plant tissue decomposition (Bao et al., 2013a, 2013b; Vane et al., 2013). Additionally, mangrove plants themselves also have a high content of phenols (Rahim et al., 2007, 2008). Phenols and low molecular organic acids, the main root exudates

---

Table 1
General physical–chemical properties of the sediments used.

pH	Total phenols	Total organic matter	Salt content	Silt content	Total S	Fe	Cd
	mg/kg	%	‰	%	%	%	mg/kg
6.88 ± 0.02	23.99 ± 4.11	8.42 ± 0.72	16.71 ± 0.46	84.1 ± 0.3	0.18 ± 0.02	4.72 ± 0.21	0.69 ± 0.04

Data are shown as Mean $\pm$ S.D. (n = 3).

of mangroves, have a great influence on the dynamic process of Cd in the soil-plant interface by impacting on the physical–chemical properties of elements (Lu et al., 2007). They function as chelators for heavy metals depending on the molecular structure (Brown et al., 1998; Michalak, 2006). Possessing the richest oxygen-containing functional groups, phenolic compounds (–OH) etc. are known to play a critical role towards persisting metal elements (Hesterberg et al., 2001) and are recognized as the main active ingredients in soils transforming the speciation of heavy metals through chelation (Hutchison et al., 2001; Karlsson and Skyllberg, 2007). The potential of these secondary metabolites for soil remediation candidates is considerable for contaminated estuary wetlands.

High sulphur, high iron, high organic matter content, nutrient-rich muddy substrates and oxygen-poor are characteristics of mangrove sediments (Clark et al., 1998; Ferreira et al., 2010). The chemical reactions of sulphur and iron are considered to be the key processes controlling the sediment chemical environment in mangroves (Kostka and Luther, 1995; Ferreira et al., 2007; Alongi, 2010). Karlsson and Skyllberg (2007) have shown that elemental sulphur significantly affected the bioavailability, migration and transformation of heavy metal in the soils containing rich organic matter. Phenolic compounds (–OH) play a critical role towards persisting metal elements, but the study showed that metals take the leading role in bonding with S-containing function groups (Hesterberg et al., 2001). AVS possessing high activity influences metal migration (Youssef and Saenger, 1998; Marchand et al., 2004) and has correlated relatively well with heavy metals (Martinez et al., 2002). Reddy and Patrick (1977) estimated that the mobility and utilisation of Cd were controlled by highly stable CdS under low redox potential (Eh), which mainly resulted from precipitation between AVS and Ex-Cd. Iron is another element which plays an important role in the biogeochemical cycle in mangrove environments. Fe oxides or hydroxides bonding heavy metals have been revealed to be the dominant fraction in the Fe rich sedimentary environment (Liu et al., 2008; Prasad and Ramanathan, 2010). Fe limits mangrove growth indirectly (Alongi, 2010) with the Fe cycle in its sediments closely linked with the sulphur cycles via microbial mediation (Kostka and Luther, 1995). Furthermore, Fe (II) and Ex-Cd compete to combine with phenols which act as chelating agents and carriers. Therefore, the Fe cycle may affect the activities of heavy metal pollutants (Marchand et al., 2006). Iron plaque on root surfaces is known as a coating (Batty et al., 2002; Tripathi et al., 2014), commonly found in mangrove plants. Pi et al. (2011) suggested that iron plaque acted as a physical 'barrier' immobilizing and co-precipitating trace metals and lowered bioavailability and absorption of trace metals in plant roots.

To our knowledge, this is one of a very few research investigations which has studied the biogeochemical behavior of Cd under the influence of phenols in mangroves. In this study, A. marina was cultivated in rhizoboxes under different Cd and phenol concentrations. The distributions of Ex-Cd, AVS and reactive solid-phase Fe (II) were examined. The aims were: (1) to assess the influence of phenols on the biogeochemistry of Cd, (2) to identify the distribution of Ex-Cd and its potential perniciousness in mangroves,

and (3) to evaluate the significance of phenols for soil remediation.

2. Materials and methods

2.1. Experimental set up

The sediment sample used in this study was collected from the mangrove forest of the Jiulong River estuary in Fujian Province, China and was pretreated by mixing and homogenizing. Different amounts of Cd were then added by using $CdCl_2$, separately as 0 (control), 1, 2 and 4 mg/kg dry weight. The treated sediments were kept fresh by adding distilled water and homogenized by mixing thoroughly each week for two months.

A laboratory rhizobox approach, (adopted from Liu et al., 2008), was improved and utilized to plant the mangrove seedlings (Fig. 1). Briefly, each rhizobox was divided into different sections by nylon cloth (500 meshes). A root bag 7 cm in height was fabricated to plant mangrove seedlings. Next to the root bag, a 2 mm-section, defined as the rhizosphere, was separated with non-rhizosphere sediment by nylon cloth. This rhizobox is characterized by accessibility, easy operation and convenience for researching the distribution and migration of elements. About 3 kg of the treated sediment was added to each rhizobox, and each treatment made in triplicate. Four A. marina seedlings obtained from the Jiulong River
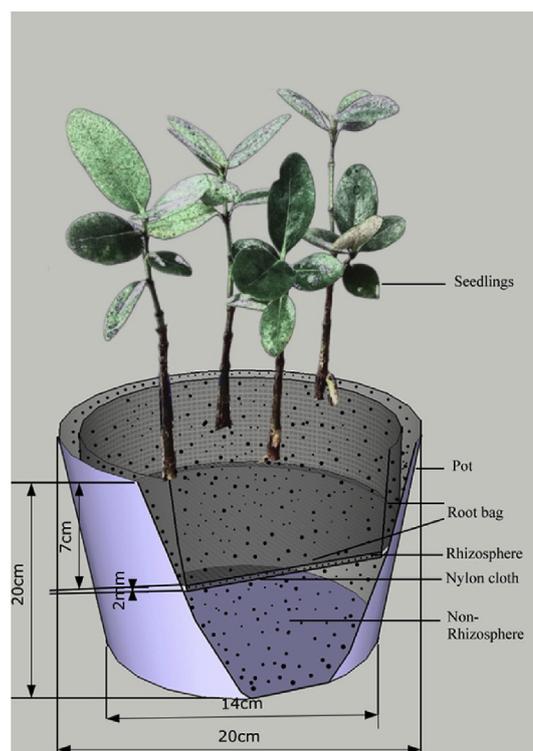

Fig. 1. Sketch of the rhizobox used in this study.

estuary were planted in each root box. Two series of phenols (0 and 15 mg/kg DW, Catechin: Gallic acid = 1:2) were added every 9 d during which Catechin and Gallic acid could decompose. Plants were grown under greenhouse conditions.

2.2. Sampling

After 45 d of cultivation, sediments within the rhizosphere and non-rhizosphere were collected separately. These were immediately sealed and stored frozen to prevent oxygen diffusion. The roots were washed out with tap water to remove sediment and kept intact. Part of the root samples were used for analysis of iron plaque and root porosity. Residual roots, all leaves and stems were separated and then freeze-dried. The dried samples were finely ground in an agate mortar for analysis.

2.3. Analysis

At harvest, the detection of root porosity was performed by the pycnometer method (Li et al., 2011). Iron plaque on fresh root surfaces was extracted according to the DCB technique of Taylor and Crowder (1983).

Sediment samples that had been stored frozen were subsampled after thawing and homogenizing under nitrogen gas (N_2). AVS was extracted adopting the cold-acid purge-and-trap method of Allen et al. (1993). Ex-Cd was extracted using the first step of sequential extraction procedure (Tessier et al., 1979). Reactive solid-phase Fe was achieved by anoxic oxalate extraction (Burton et al., 2007) and the ferrous iron content in extracts was analyzed by the 1, 10-phenanthroline method (Phillips and Lovley, 1987).

Plant samples were digested as described by Soto-Jimenez and Paez-Osuna (2001). Reagent blanks and standard references of soil (GBW-070310), plant material (GBW-07603) (from the National Research Center for Standards in China) were included to verify the accuracy and precision of the digestion procedure and subsequent analysis. All reagents were Merck analytical grade or Suprapur quality and all materials (bottles, filters etc.) were acid-cleaned (14% (v/v) nitric acid) and rinsed with deionized water prior to use. Deionized water was used in preparing stock solutions and in each step of the procedures. Metal concentrations in the samples were determined by flame atomic absorption spectrometry (AAS, Model AA-6800, Shimadzu, Kyoto) with 98–101% percentage recovery for Cd and 99–103% for Fe. The concentration factor was calculated as the ratio of the metal concentrations in the samples (in micrograms per gram dry weight (DW)) and the concentrations in the solution (in milligrams per litre).

To assess the capabilities of accumulating and transporting Cd in the plant, the bioconcentration factor (BCF) and translocation factor (TF) were calculated and analyzed.

2.4. Statistical analysis

The data were analyzed using a statistical package, SPSS version 13.0. Two-way analysis of variance was carried out. All results presented and discussed are based on mean values and standard deviation (S.D.) in triplicate, and were tested by the Ducan and least significant difference (LSD).

3. Results

Eh variation depending on Cd treatment was detected under phenol treatment in the sediments (Fig. 2). Eh apparently lowered response to phenols in both the rhizosphere and non-rhizosphere; at the same time, Eh decreased depending on Cd concentration
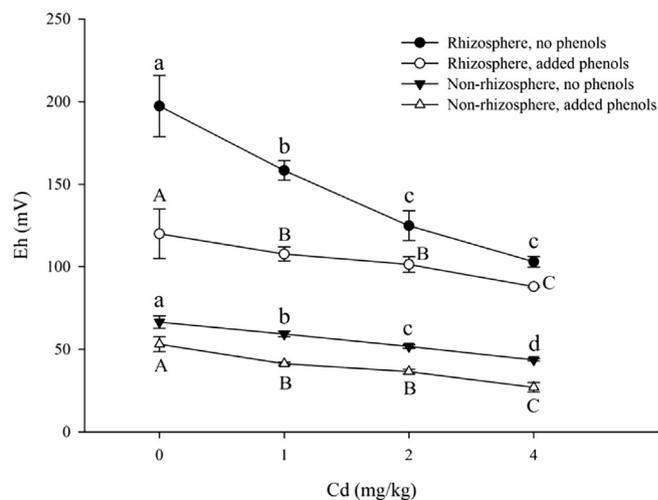

Fig. 2. Eh change trend in rhizosphere and non-rhizosphere. Values with the same letters are equivalent; different letters denote significant differences ($p < 0.05$) based on ANOVAs.

($p < 0.01$). It is evident that Eh in the rhizosphere is higher than that in the non-rhizosphere ($p < 0.01$).

In the rhizosphere, Ex-Cd had clearly increased after phenol treatment (Fig. 3a, $p < 0.05$). Ex-Cd concentrations were significantly higher under the rising Cd treatment ($p < 0.01$). In the non-rhizosphere (Fig. 3a), the results were similar to the rhizosphere ($p < 0.05$). Except at exposure to low concentration Cd (1 mg/kg), Ex-Cd concentrations in the rhizosphere were much higher than that in the non-rhizosphere ($p < 0.05$).

Phenol treatment facilitated formation of AVS in the mangrove sediments (Fig. 3b, $p < 0.05$). AVS concentration in the non-rhizosphere was significantly higher than that in the rhizosphere ($p < 0.01$). In response to exposure to Cd at low concentration, the content of AVS was lower than the blank, but increased depending on rising Cd treatment and presented a statistically significant positive correlation with it ($p < 0.01$).

Under phenol and Cd treatment, reactive solid-phase Fe (II) presented an increasing trend (Fig. 3c, $p < 0.05$). It proved most obvious in the non-rhizosphere. Compared with the rhizosphere, reactive solid-phase Fe (II) concentration was higher in the non-rhizosphere because the performance of ROL (radial oxygen loss) caused Fe (II) oxidation in rhizosphere. Reactive solid-phase Fe (II) showed a rising trend after the first decrease with Cd applied in the rhizosphere; whereas a sustained upward trend was shown in the non-rhizosphere. The trends presented a significant positive correlation with Cd treatment ($p < 0.05$).

Cd and Fe concentration significantly increased by Cd treatment ($p < 0.01$) in DCB extracts (Fig. 4). Fe concentration gave a statistically significantly positive correlation with Cd concentration in DCB extracts ($p < 0.01$). Concentration of iron increased when phenols were added ($p < 0.01$). In turn, Cd concentration lowered dramatically in response to exposure to phenols ($p < 0.01$).

Fig. 5, shows root porosity decreased and negatively correlated ($p < 0.01$) with the Cd treatment. It also decreased when provided with phenols ($p < 0.01$).

In response to phenols, Cd concentration lowered in roots and stems; while the opposite occurred in leaves (Table 2). The concentrations of Cd in roots, stems and leaves demonstrate a highly significant positive correlation with Cd treatment ($p < 0.01$). Inversely, BCF-Cd and TF-Cd in plants (Table 3) decreased continuously with increasing Fe and Cd contents in DCB-extracts and Cd treatment in sediments and showed significant negative correlation with them ($p < 0.01$). Phenols enabled BCF-Cd and TF-Cd to reduce
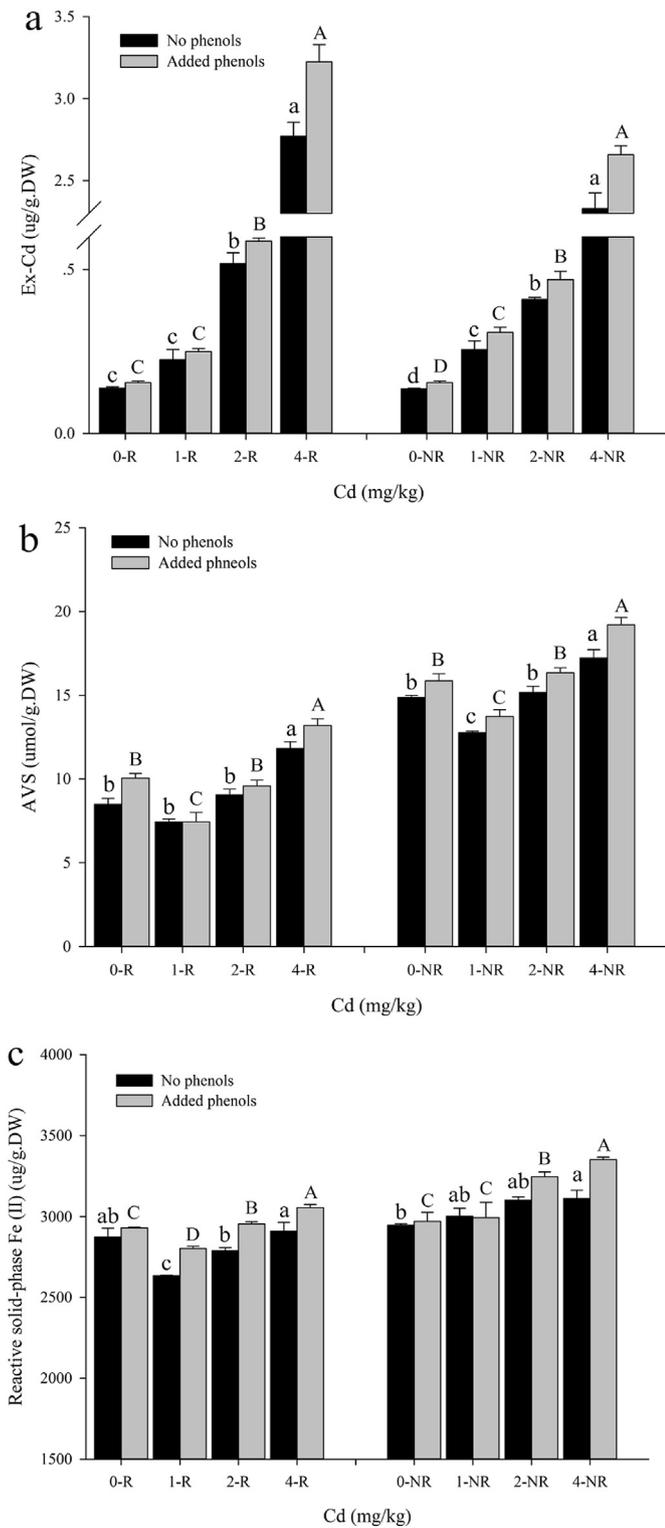
Fig. 3. Ex-Cd (a), AVS (b) and Reactive solid-phase Fe (II) (c) concentration in sediments. Values with the same letters are equivalent; different letters denote significant differences ($p < 0.05$) based on ANOVAs. 0/1/2/4-R and 0/1/2/4-NR mean Cd treatment (0/1/2/4 mg/kg DW) in sediments (rhizosphere and non-rhizosphere).

Fig. 4. Cd (a) and Fe (b) concentration in DCB-extracts. Values with the same letters are equivalent; different letters denote significant differences ($p < 0.05$) based on ANOVAs.
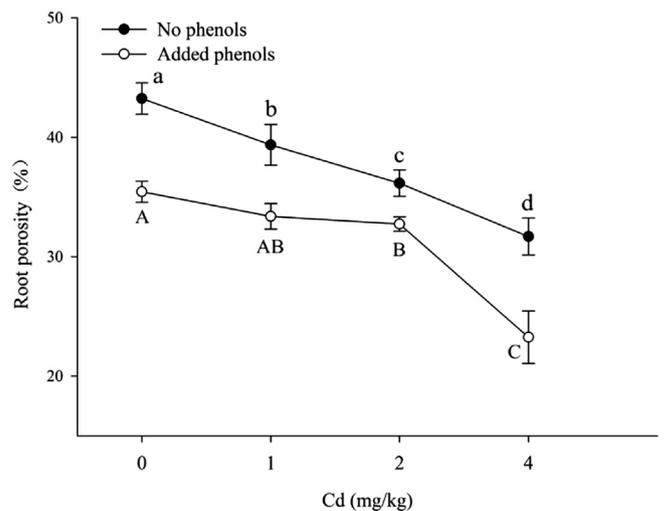
Fig. 5. Distribution of root porosity. Values with the same letters are equivalent; different letters denote significant differences ($p < 0.05$) based on ANOVAs.

in both roots and stems, but not in leaves (Table 3). The accumulation and distribution of Cd in plants showed a decreasing order of: roots > stems > leaves. In Table 2, data have revealed an obvious increase of Fe concentration in plants under phenol treatment. Fe concentration showed a highly significant positive correlation with
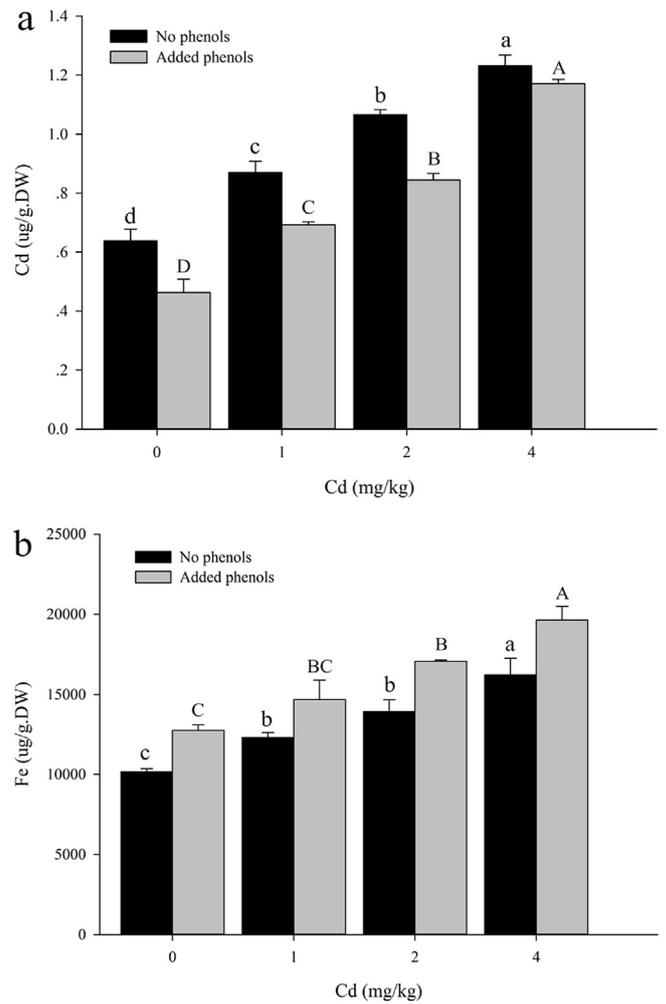
Cd treatment, especially in roots ($p < 0.01$). BCF-Fe and TF-Fe in plants gave a significant positive correlation with Fe and Cd contents in DCB-extracts ($p < 0.01$), except TF-Fe in stems exposed to Cd stress alone. Phenols exerted positive effects for enrichment and translocation of Fe in plants (Table 3).

4. Discussion

Exterior environmental factors (e.g., heavy metals, organic acid and sulphide) can change root anatomical structure (Armstrong and Armstrong, 2001, 2005) or morphology (Taggart et al., 2009), such as root porosity (Visser et al., 2000; Li et al., 2011), and then regulate ROL from roots. ROL has a major impact on the Eh in sediments. Results from a previous researcher (Cheng et al., 2010), as well as the present study proved reduced root porosity in response to heavy metal stress (Fig. 5). This may partly explain why Eh was reduced with the concentrations of Cd. Olsen et al. (1981) showed that phenols are characteristics of reducibility. Substitution patterns on the B-ring and hydroxyl substituents on the A-ring are the determinant of phenol reducibility in their molecular structure (Arora et al., 1998). Therefore, when phenols were provided here, Eh showed a decreasing trend in sediments (Fig. 2). Eh and pH most affect the metal chemical process and bioavailability in soils. The absorption capacity of Cd decreased with rising Eh in soils and desorption ability increased by increasing reduction capacity after Cd was adsorbed (Ge et al., 2006). Phenols induce strong acidification in sediments and behave as desorption and solubilization agents, which desorb Cd^{2+} from sediment particles (Olsen et al., 1981) dissolving Cd and other solutes (Charlatchka and Cambier, 2000). Phenols prompt Cd to form Ex-Cd (Charlatchka and Cambier, 2000) with Ex-Cd concentration rising in the rhizosphere and non-rhizosphere (Fig. 3a). In this study, oxygen and Eh gradients occurred between the rhizosphere and non-rhizosphere, which made AVS content in the non-rhizosphere higher than that in the rhizosphere (Fig. 3b). This induced Cd^{2+} immobilization and reduced activity of Cd. Alongside, root exudates in the rhizosphere dissolved and chelated Cd, affected species distribution of Cd (Lu et al., 2007; Cesco et al., 2010, 2012), offered similar benefits to exogenous phenols, and caused chelating Cd and organic content higher in the rhizosphere, so that Ex-Cd in the rhizosphere was more than the non-rhizosphere (Fig. 3a, $p < 0.05$). Lin (2010) found that exchangeable Cu concentration in the rhizosphere of paddy soils was higher than that in non-rhizosphere, which accords with our Cd results. Phenols catalyze Cd which enhances its activity and then disturbs the "source-sink" balance by converting it into a "source" in the mangrove sediments.

Phenols are characteristic of solubility, chelation and reduction mechanisms (Olsen et al., 1981; Arora et al., 1998), facilitating the reducing process of SO_4^{2-} to S^{2-}. As a response to phenols exposure, some slightly soluble sulphides (FeS_2, CdS etc.) will resolve to develop S^{2-} and Fe^{2+}/Cd^{2+} through a series of chemical reactions under the common actions of solubility, chelation and reducibility of phenols. This may lead to AVS and Ex-Cd concentrations increasing (Fig. 3b, a). Under reducing conditions, sulphate is prone to reduction reaction. ROL formed micro aerophilic zones and lowered reducibility in the rhizosphere, sulphur oxidation occurred in the rhizosphere; while in the non-rhizosphere, sulphur reduction reaction occurred. Accordingly, AVS content was greatly higher in the non-rhizosphere than the rhizosphere (Fig. 3b, $p < 0.01$). As the most active speciation of sulphur, AVS greatly affects the solubility and bioavailability of Cd. It can be used as a primary source for S^{2-} ligands and generates CdS through substitution reaction, which decreases the activity of Cd in reducing conditions (Huerta-Diaz et al., 1998). Because of the development of CdS, low concentration of Cd stress lead to decreasing AVS content. Decreasing Eh provided a stronger reducing environment for AVS and stimulated its production, resulting in AVS concentration increasing in the sediments. Phenols enhanced both concentrations of Ex-Cd and AVS in sediments. AVS concentration affects the speciation distribution of Cd, and vice versa. The speciation distribution of sulphur is impacted by Cd concentration in the mangrove sediments.

Iron-manganese oxides or hydroxides are known as strong adsorbers scavenging most of the heavy metals. Redox conditions cause the change of bonds between metal and iron-manganese oxides or hydroxides, resulting in the mobility of metals (Chuan et al., 1996; Davranche and Bollinger, 2000). The concentration of Cd greatly increases and reveals significant positive correlation with Fe (II) concentration when Eh lowers from $+325$ mV to -100 mV (Chuan et al., 1996). The mobility of divalent trace metals was facilitated by reducing conditions which closely followed the redox potential of Fe and Mn (Charlatchka and Cambier, 2000); part of the Fe oxide here was reduced and released Cd^{2+} from its bonding causing increase of Fe (II) and Ex-Cd contents (Fig. 3c, a). Indeed, soluble organic acids prevent readsorption and precipitation of metal, thus the reactive solid-phase Fe (II) and Ex-Cd rose under phenol treatment (Fig. 3c, a). Arora et al. (1998) proved that the substitution patterns especially affected antioxidant potencies in phenol molecular structure: hydroxyl substituents in a catechol structure on the A-ring were able to compensate substitution on the B-ring and become a larger determinant of phenols antioxidant activity when substitution on the B-ring was abolished. In this study, the strong antioxidant potency of phenols increased concentrations of reactive solid-phase Fe (II) and AVS in the rhizosphere and non-rhizosphere sediments (Fig. 3c, b).

Previous research showed that iron plaque on the root surface acted as a physical 'barrier' (Pi et al., 2011b). Accumulation of Fe in DCB-extracts here presented a significant positive correlation with Cd treatment (Fig. 4b, $p < 0.01$). Decreasing TF-Cd in leaves and stems provide evidence of iron plaque preventing heavy metals transporting into the plants (Table 3). Phenols increased the concentration of Fe (II) in sediments (Fig. 3c), and more Fe (II) was oxidized (ROL was an important factor for Fe (II) oxidation (Pi et al., 2010)) and immobilized in the root surface, increasing the quantity of iron plaque. The solubilization and desorption (Stingu et al., 2012) of phenols enable Cd desorption from iron plaque and hamper re-adsorption or precipitation of metals. When provided with phenols, the concentration of Cd in DCB-extracts decreased (Fig. 4a). Visibly, phenols were not conducive to Cd accumulation in iron plaque.

Reddy and Patrick (1977) suggested that Eh affected the solubility and bioavailability of flooded soil Cd: the uptake of Cd decreased when the Eh was lower. This observation supports our work that Cd accumulation decreased in plants as a response to phenols which led to Eh reduction (Tables 2 and 3). A significant negative correlation existed between BCF-Cd, TF-Cd in plants and Fe, Cd in DCB-extracts ($p < 0.01$). Iron plaque precipitated some of the Cd and prevented adsorption and enrichment of Cd in plants (Tables 2 and 3); it acted as a reservoir to immobilize Cd (Pi et al., 2011a). Also, the solubilization and desorption of phenols hinder absorption of Cd in iron plaque (Fig. 4), which closed the "first door" for Cd access and decreasing the root uptake. Enhancing the phenols played a positive role in plant protection and reduced the uptake, accumulation and translocation of Cd in roots and stems. In leaves, Cd may substitute Fe to participate in photosynthesis, the synthesis of chlorophyll, amino acid and protein; simultaneously Cd^{2+} competed with Fe (II) for chelating with phenols and transporting to the cytomembrane. This facilitated the movement of Cd^{2+} in plants in which the phenols act

Table 2
Cd and Fe accumulation in the different tissues of *A. marina* seedlings.

Treatment		Roots		Steams		Leaves	
		Cd (ug/g)	Fe (mg/g)	Cd (ug/g ($\times 10^{-1}$))	Fe (mg/g ($\times 10^{-1}$))	Cd (ug/g ($\times 10^{-1}$))	Fe (mg/g ($\times 10^{-1}$))
Cd_0	Ph_0	1.75 ± 0.19d	49.76 ± 8.21d	12.48 ± 0.84b	2.28 ± 0.17b	1.38 ± 0.09b	1.55 ± 0.13d
	Ph_1	1.00 ± 0.26d	62.29 ± 1.83d	5.74 ± 0.06b	2.83 ± 0.30b	3.55 ± 0.14b	2.17 ± 0.14b
Cd_1	Ph_0	2.15 ± 0.11c	62.51 ± 1.79c	13.29 ± 1.18b	3.07 ± 0.4a	3.61 ± 0.09 ab	1.83 ± 0.05c
	Ph_1	1.51 ± 0.29c	65.10 ± 0.87c	7.57 ± 0.10b	3.30 ± 0.28b	3.65 ± 0.08b	2.43 ± 0.01b
Cd_2	Ph_0	2.98 ± 0.18b	74.22 ± 9.07b	10.67 ± 2.63c	3.16 ± 0.09a	3.62 ± 0.12 ab	2.31 ± 0.16b
	Ph_1	2.07 ± 0.02b	79.91 ± 11.66b	8.329 ± 0.06b	4.62 ± 0.64a	5.40 ± 0.07a	3.87 ± 0.62a
Cd_4	Ph_0	6.17 ± 0.35a	83.22 ± 3.97a	25.39 ± 1.29a	3.36 ± 0.76a	5.74 ± 0.03a	3.14 ± 0.18a
	Ph_1	4.70 ± 0.02a	91.99 ± 2.08a	17.11 ± 0.27a	5.42 ± 0.37a	6.24 ± 0.12a	4.10 ± 0.29a
Analysis of variance	Cd	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
	Ph	<0.001	<0.001	<0.001	<0.001	0.012	<0.001
	Cd × Ph	0.007	ns	0.004	0.008	ns	0.022

Note: Cd_0, Cd_1, Cd_2 and Cd_4 indicate Cd treatments concentration 0, 1, 2 and 4 mg/kg DW respectively. Ph means phenols, Ph_0 and Ph_1 mean "no phenols" and "add phenols" respectively. Data are shown as Mean ± S.D. (n = 3). "ns" mean no significance ($p > 0.05$). Values with the same letters are equivalent; different letters denote significant differences ($p < 0.05$) based on ANOVAs. All analysis results were carried out by a two-way ANOVA.

Table 3
Bioconcentration factor (BCF) and translocation factor (TF) of Cd and Fe in *A. marina* seedlings.

Parts		Treatment					Analysis of variance		
		Ph	Cd_0	Cd_1	Cd_2	Cd_4	Cd	Ph	Cd × Ph
Roots	BCF-Cd	Ph_0	2.19 ± 0.07a	1.19 ± 0.06b	1.06 ± 0.02c	1.28 ± 0.07b	<0.001	<0.001	0.001
		Ph_1	1.25 ± 0.18a	0.84 ± 0.09 ab	0.74 ± 0.01b	0.97 ± 0.01 ab			
	BCF-Fe	Ph_0	1.28 ± 0.19d	1.68 ± 0.01c	2.01 ± 0.11b	2.25 ± 0.10a	<0.001	<0.001	0.007
		Ph_1	1.68 ± 0.05d	1.77 ± 0.01c	2.16 ± 0.01b	2.51 ± 0.03a			
Stems	BCF-Cd ($\times 10^{-1}$)	Ph_0	15.58 ± 1.05a	7.39 ± 0.91b	3.88 ± 0.05d	5.06 ± 0.08c	<0.001	<0.001	<0.001
		Ph_1	7.17 ± 0.71a	4.30 ± 0.53b	3.14 ± 0.11c	3.15 ± 1.76c			
	TF-Cd ($\times 10^{-1}$)	Ph_0	7.40 ± 0.03a	6.21 ± 0.33b	4.58 ± 0.40c	4.12 ± 0.21d	<0.001	<0.001	<0.001
		Ph_1	5.71 ± 0.53a	5.06 ± 0.44a	4.00 ± 0.32b	3.34 ± 0.20b			
	BCF-Fe ($\times 10^{-3}$)	Ph_0	6.02 ± 0.30c	7.87 ± 0.36b	8.53 ± 0.23b	9.87 ± 0.68a	<0.001	<0.001	0.033
		Ph_1	7.91 ± 0.46c	9.35 ± 0.61c	12.82 ± 1.53b	14.97 ± 0.58a			
	TF-Fe ($\times 10^{-3}$)	Ph_0	4.58 ± 0.99a	4.54 ± 0.01a	4.26 ± 0.01a	4.56 ± 0.32a	ns	<0.001	0.029
		Ph_1	4.78 ± 0.10c	5.32 ± 0.21b	5.48 ± 0.27b	6.16 ± 0.14a			
Leaves	BCF-Cd ($\times 10^{-1}$)	Ph_0	1.75 ± 0.08a	1.58 ± 0.31 ab	1.29 ± 0.42 ab	1.19 ± 0.11b	<0.001	<0.001	<0.001
		Ph_1	4.38 ± 0.40a	2.03 ± 0.45b	1.80 ± 0.12b	1.30 ± 0.23c			
	TF-Cd ($\times 10^{-1}$)	Ph_0	2.06 ± 0.32a	1.67 ± 0.63 ab	1.22 ± 0.48 ab	0.93 ± 0.12b	<0.001	<0.001	0.011
		Ph_1	3.97 ± 0.66a	2.66 ± 0.03b	2.45 ± 0.17b	1.45 ± 0.12c			
	BCF-Fe ($\times 10^{-3}$)	Ph_0	4.06 ± 0.20d	4.93 ± 0.14c	6.23 ± 0.44b	8.49 ± 0.49a	<0.001	0.001	0.039
		Ph_1	5.86 ± 0.37c	6.55 ± 0.03c	9.49 ± 0.23b	11.071 ± 0.78a			
	TF-Fe ($\times 10^{-3}$)	Ph_0	2.72 ± 0.12c	2.92 ± 0.08c	3.22 ± 0.11b	3.78 ± 0.22a	<0.001	<0.001	ns
		Ph_1	3.49 ± 0.31b	3.73 ± 0.04b	4.55 ± 0.77a	4.45 ± 0.28a			

Note: Cd_0, Cd_1, Cd_2 and Cd_4 indicate Cd treatments concentration 0, 1, 2 and 4 mg/kg DW respectively. Ph means phenols, Ph_0 and Ph_1 mean "no phenols" and "add phenols" respectively. Data are shown as Mean ± S.D. (n = 3). "ns" mean no significance ($p > 0.05$). "BCF-Cd/BCF-Fe" means bioconcentration factor of Cd/Fe, and "TF-Cd/TF-Fe" represents translocation factor of Cd/Fe. Values with the same letters are equivalent; different letters denote significant differences ($p < 0.05$) based on ANOVAs. All analysis results were carried out by a two-way ANOVA.

as a carrier. They both encourage Cd enrichment and translocation and hence Cd concentration. BCF-Cd and TF-Cd in leaves were higher under phenol treatment (Table 3). Significant positive correlation between BCF-Fe, TF-Fe in plants and Fe, Cd contents in DCB-extracts was found. This illustrated that accumulation of Fe as iron plaque enhanced the uptake of Fe. In plants, the increase of Fe derived from the increase of Fe in DCB-extracts and Fe (II) in the rhizosphere when phenols were added (Table 2). Fe concentrations showed a greatly positive correlation with Cd concentration in plants ($p < 0.01$). These all further demonstrated the viewpoint of a competition between Cd^{2+} and Fe (II) (Harikumar and Nasir, 2010).

5. Conclusion

Cd was activated as a response to phenol treatment which increased activity of Cd in mangrove sediments, and especially in the rhizosphere. This result implies that phenols disturbed the "source-sink" balance of Cd in mangrove sediments. It appears that phenol treatment resulted in the increase of Cd activity. But the concentration of Cd clearly decreased in the plants themselves, accounting for Cd accumulation decreasing in iron plaque, and the competition between Fe (II) and Cd^{2+} impeding Cd uptake in *A. marina* seedling roots. As for the biogeochemical behavior of Cd in mangrove sediments, it is an arresting discovery that phenols disturbed the "source-sink" balance of Cd and turned it into a "source". Meanwhile, phenols are shown to play a critical role in protecting plants from Cd toxication in mangroves. The potential of phenols as soil remediation candidates can be considerable for contaminated estuary wetlands. Phenols facilitated Fe (II) production in sediments and iron accumulation in iron plaque which was favourable for Fe absorption in plant roots. Therefore, they alleviated the limit of Fe to mangrove plant growth. However, more focus is needed on research into the molecular mechanism of phenols in protecting mangroves from heavy metal contamination.

Acknowledgments

References

Alongi, D.M., 2010. Dissolved iron supply limits early growth of estuarine mangroves. Ecology 91, 3229–3241.

Allen, H.E., Fu, G.M., Deng, B.L., 1993. Analysis of acid-volatile sulfide (AVS) and simultaneously extracted metals (SEM) for the estimation of potential toxicity in Aquatic sediments. Environ. Toxicol. and Chem. 12, 1441–1453.

Armstrong, J., Armstrong, W., 2001. Rice and Phragmites: effects of organic acids on growth, root permeability, and radial oxygen loss to the rhizosphere. Am. J. Bot. 88, 1359–1370.

Armstrong, J., Armstrong, W., 2005. Rice: sulfide-induced barriers to root radial oxygen loss, Fe2+ and water uptake, and lateral root emergence. Ann. Bot. 96, 625–638.

Arora, A., Nair, M.G., Strasburg, G.M., 1998. Structure-activity relationships for antioxidant activities of a series of flavonoids in a liposomal system. Free Radic. Biol. Med. 24, 1355–1363.

Bao, H.Y., Wu, Y., Tian, L.X., Zhang, J., Zhang, G.L., 2013a. Sources and distributions of terrigenous organic matter in a mangrove fringed small tropical estuary in South China. Acta Oceanol. Sin. 32, 18–26.

Bao, H.Y., Wu, Y., Unger, D., Du, J.Z., Herbeck, L.S., Zhang, J., 2013b. Impact of the conversion of mangroves into aquaculture ponds on the sedimentary organic matter composition in a tidal flat estuary (Hainan Island, China). Cont. Shelf Res. 57, 82–91.

Batty, L.C., Baker, A.J.M., Wheeler, B.D., 2002. Aluminium and phosphate uptake by Phragmites australis: the role of Fe, Mn and Al root plaques. Ann. Bot. 89, 443–449.

Brown, J.E., Khodr, H., Hider, R.C., Rice-Evans, C.A., 1998. Structural dependence of flavonoid interactions with Cu2+ ions: implications for their antioxidant properties. Biochem. J. 330, 1173–1178.

Burton, E.D., Bush, R.T., Sullivan, L.A., Mitchell, D.R.G., 2007. Reductive transformation of iron and sulfur in schwertmannite-rich accumulations associated with acidified coastal lowlands. Geochim. Cosmochim. Acta 71, 4456–4473.

Cesco, S., Mimmo, T., Tonon, G., Tomasi, N., Pinton, R., Terzano, R., Neumann, G., Weisskopf, L., Renella, G., Landi, L., Nannipieri, P., 2012. Plant-borne flavonoids released into the rhizosphere: impact on soil bio-activities related to plant nutrition. A review. Biol. Fertil. Soils 48, 123–149.

Cesco, S., Neumann, G., Tomasi, N., Pinton, R., Weisskopf, L., 2010. Release of plant-borne flavonoids into the rhizosphere and their role in plant nutrition. Plant Soil 329, 1–25.

Charlatchka, R., Cambier, P., 2000. Influence of reducing conditions on solubility of trace metals in contaminated soils. Water Air Soil Pollut. 118, 143–167.

Cheng, H., Liu, Y., Tam, N.F.Y., Wang, X., Li, S.Y., Chen, G.Z., Ye, Z.H., 2010. The role of radial oxygen loss and root anatomy on zinc uptake and tolerance in mangrove seedlings. Environ. Pollut. 158, 1189–1196.

Chou, C.L., Paon, L.A., Moffatt, J.D., Buzeta, M.I., Fenton, D., Rutherford, R.J., 2004. Distribution of contaminants in biota and sediments in the Musquash Estuary, Atlantic Canada, marine protected area site initiative and contaminant exclusion zone. Mar. Pollut. Bull. 48, 884–893.

Chuan, M.C., Shu, G.Y., Liu, J.C., 1996. Solubility of heavy metals in a contaminated soil: effects of redox potential and pH. Water Air Soil Pollut. 90, 543–556.

Clark, M.W., McConchie, D., Lewis, D.W., Saenger, P., 1998. Redox stratification and heavy metal partitioning in Avicennia-dominated mangrove sediments: a geochemical model. Chem. Geol. 149, 147–171.

Davranche, M., Bollinger, J.C., 2000. Heavy metals desorption from synthesized and natural iron and manganese oxyhydroxides: effect of reductive conditions. J. Colloid Interface Sci. 227, 531–539.

Du, J.N., Yan, C.L., Li, Z.D., 2013. Formation of iron plaque on mangrove Kandalar. Obovata (SL) root surfaces and its role in cadmium uptake and translocation. Mar. Pollut. Bull. 74, 105–109.

Ferreira, T.O., Otero, X.L., de Souza, V.S., Vidal-Torrado, P., Macias, F., Firme, L.P., 2010. Spatial patterns of soil attributes and components in a mangrove system in Southeast Brazil (Sao Paulo). J. Soils Sediment 10, 995–1006.

Ferreira, T.O., Otero, X.L., Vidal-Torrado, P., Macias, F., 2007. Effects of bioturbation by root and crab activity on iron and sulfur biogeochemistry in mangrove substrate. Geoderma 142, 36–46.

Ge, Y., Li, Y., Zhou, Q., 2006. Preliminary investigation on adsorption and desorption of Cd in soil with different reduction state (In China). Ecol. Environ. 15, 730–734.

Harikumar, P.S., Nasir, U.P., 2010. Ecotoxicological impact assessment of heavy metals in core sediments of a tropical estuary. Ecotoxicol. Environ. Safe 73, 1742–1747.

Hesterberg, D., Chou, J.W., Hutchison, K.J., Sayers, D.E., 2001. Bonding of Hg(II) to reduced organic, sulfur in humic acid as affected by S/Hg ratio. Environ. Sci. Technol. 35, 2741–2745.

Huerta-Diaz, M.A., Tessier, A., Carignan, R., 1998. Geochemistry of trace metals associated with reduced sulfur in freshwater sediments. Appl. Geochem. 13, 213–233.

Hutchison, K.J., Hesterberg, D., Chou, J.W., 2001. Stability of reduced organic sulfur in humic acid as affected by aeration and pH. Soil Sci. Soc. Am. J. 65, 704–709.

Karlsson, T., Skyllberg, U., 2007. Complexation of zinc in organic soils - EXAFS evidence for sulfur associations. Environ. Sci. Technol. 41, 119–124.

Kostka, J.E., Luther, G.W., 1995. Seasonal cycling of Fe in salt-marsh sediments. Biogeochemistry 29, 159–181.

Li, H., Ye, Z.H., Wei, Z.J., Wong, M.H., 2011. Root porosity and radial oxygen loss related to arsenic tolerance and uptake in wetland plants. Environ. Pollut. 159, 30–37.

Lin, H.R., 2010. Microbe mediated molecular speciation and transformation of sulfur and heavy metals in paddy soil. Zhejiang University.

Liu, J.C., Yan, C.L., Spencer, K.L., Zhang, R.F., Lu, H.L., 2010. The distribution of acid-volatile sulfide and simultaneously extracted metals in sediments from a mangrove forest and adjacent mudflat in Zhangjiang Estuary, China. Mar. Pollut. Bull. 60, 1209–1216.

Liu, J.C., Yan, C.L., Zhang, R.F., Lu, H.L., Qin, G.Q., 2008. Speciation changes of cadmium in mangrove (Kandelia candel (L.)) rhizosphere sediments. Bull. Environ. Contam. Toxicol. 80, 231–236.

Lu, H.L., Yan, C.L., Liu, J.C., 2007. Low-molecular-weight organic acids exuded by Mangrove (Kandelia candel (L.) Druce) roots and their effect on cadmium species change in the rhizosphere. Environ. Exp. Bot. 61, 159–166.

MacFarlane, G.R., Pulkownik, A., Burchett, M.D., 2003. Accumulation and distribution of heavy metals in the grey mangrove, Avicennia marina (Forsk.)Vierh.: biological indication potential. Environ. Pollut. 123, 139–151.

Marchand, C., Baltzer, F., Lallier-Verges, E., Alberic, P., 2004. Pore-water chemistry in mangrove sediments: relationship with species composition and developmental stages (French Guiana). Mar. Geol. 208, 361–381.

Marchand, C., Lallier-Vergès, E., Baltzer, F., Albéric, P., Cossa, D., Baillif, P., 2006. Heavy metals distribution in mangrove sediments along the mobile coastline of French Guiana. Mar. Chem. 98, 1–17.

Martinez, C.E., McBride, M.B., Kandianis, M.T., Duxbury, J.M., Yoon, S.J., Bleam, W.F., 2002. Zinc-sulfur and cadmium-sulfur association in metalliferous peats evidence from spectroscopy, distribution coefficients, and phytoavailability. Environ. Sci. Technol. 36, 3683–3689.

Michalak, A., 2006. Phenolic compounds and their antioxidant activity in plants growing under heavy metal stress. Pol. J. Environ. Stud. 15, 523–530.

Olsen, R.A., Bennett, J.H., Blume, D., Brown, J.C., 1981. Chemical aspects of the Fe stress response mechanism in tomatoes. J. Plant Nutr. 3, 905–921.

Phillips, E.J.P., Lovley, D.R., 1987. Determination of Fe (III) and Fe (II) in oxalate extracts of sediment. Soil Sci. Soc. Am. J. 51, 938–941.

Pi, N., Tam, N., Wong, M., 2011a. Formation of iron plaque on mangrove roots receiving wastewater and its role in immobilization of wastewater-borne pollutants. Mar. Pollut. Bull. 63, 402–411.

Pi, N., Tam, N.F.Y., Wong, M.H., 2010. Effects of wastewater discharge on formation of Fe plaque on root surface and radial oxygen loss of mangrove roots. Environ. Pollut. 158, 381–387.

Pi, N., Tam, N.F.Y., Wong, M.H., 2011b. Formation of iron plaque on mangrove roots receiving wastewater and its role in immobilization of wastewater-borne pollutants. Mar. Pollut. Bull. 63, 402–411.

Prasad, M.B.K., Ramanathan, A.L., 2010. Characterization of phosphorus fractions in the sediments of a tropical intertidal mangrove ecosystem. Wetl. Ecol. Manag. 18, 165–175.

Rahim, A.A., Rocca, E., Steinmetz, J., Kassim, M.J., Adnan, R., Ibrahim, M.S., 2007. Mangrove tannins and their flavanoid monomers as alternative steel corrosion inhibitors in acidic medium. Corros. Sci. 49, 402–417.

Rahim, A.A., Rocca, E., Steinmetz, J., Kassim, M.J., Ibrahim, M.S., Osman, H., 2008. Antioxidant activities of mangrove Rhizophora apiculata bark extracts. Food Chem. 107, 200–207.

Reddy, C.N., Patrick, W.H., 1977. Effect of Redox Potential and pH on the Uptake of Cadmium and Lead by Rice Plants1. J. Environ. Qual. 6, 259–262.

Silva, C., da Silva, A.P., de Oliveira, S.R., 2006. Concentration, stock and transport rate of heavy metals in a tropical red mangrove, Natal, Brazil. Mar. Chem. 99, 2–11.

Soto-Jimenez, M.F., Paez-Osuna, F., 2001. Distribution and normalization of heavy metal concentrations in mangrove and lagoonal sediments from Mazatlan Harbor (SE Gulf of California). Estuar. Coast. Shelf. Sci. 53, 259–274.

Stingu, A., Volf, I., Popa, V.I., Gostin, I., 2012. New approaches concerning the utilization of natural amendments in cadmium phytoremediation. Ind. Crop. Prod. 35, 53–60.

Taggart, M.A., Mateo, R., Charnock, J.M., Bahrami, F., Green, A.J., Meharg, A.A., 2009. Arsenic rich iron plaque on macrophyte roots - an ecotoxicological risk? Environ. Pollut. 157, 946–954.

Taylor, G.J., Crowder, A.A., 1983. Use of the DCB technique for extraction of hudrous iron-oxides from roots of wetland plants. Am. J. Bot. 70, 1254–1257.

Tam, N.F.Y., Wong, Y.S., 1997. Accumulation and distribution of heavy metals in a simulated mangrove system treated with sewage. Hydrobiologia 352, 67–75.

Tessier, A., Campbell, P.G.C., Bisson, M., 1979. Sequential extraction procedure for the speciation of particulate trace metals. Anal. Chem. 51, 844–851.

Tripathi, R.D., Tripathi, P., Dwivedi, S., Kumar, A., Mishra, A., Chauhan, P.S., Norton, G.J., Nautiyal, C.S., 2014. Roles for root iron plaque in sequestration and uptake of heavy metals and metalloids in aquatic and wetland plants. Metallomics 6, 1789–1800.

Vane, C.H., Kim, A.W., Moss-Hayes, V., Snape, C.E., Diaz, M.C., Khan, N.S., Engelhart, S.E., Horton, B.P., 2013. Degradation of mangrove tissues by arboreal termites (Nasutitermes acajutlae) and their role in the mangrove C cycle (Puerto Rico): chemical characterization and organic matter provenance using bulk delta C-13, C/N, alkaline CuO oxidation-GC/MS, and solid-state C-13 NMR. Geochem. Geophys. Geosyst. 14, 3176–3191.

Visser, E.J.W., Colmer, T.D., Blom, C., Voesenek, L., 2000. Changes in growth, porosity, and radial oxygen loss from adventitious roots of selected mono- and dicotyledonous wetland species with contrasting types of aerenchyma. Plant Cell Environ. 23, 1237–1245.

Youssef, T., Saenger, P., 1998. Photosynthetic gas exchange and accumulation of phytotoxins in mangrove seedlings in response to soil physico-chemical characteristics associated with waterlogging. Tree Physiol. 18, 317–324.