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Iron plaque formation and heavy metal uptake in *Spartina alterniflora* at different tidal levels and waterlogging conditions

Yan Xu, Xiangli Sun, Qiqiong Zhang, Xiuzhen Li, Zhongzheng Yan*

State Key Laboratory of Estuarine and Coastal Researches, East China Normal University, Shanghai, China

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ABSTRACT

Tidal flat elevation in the estuarine wetland determines the tidal flooding time and flooding frequency, which will inevitably affect the formation of iron plaque and accumulations of heavy metals (HMs) in wetland plants. The present study investigated the formation of iron plaque and HM's (copper, zinc, lead, and chromium) accumulation in *S. alterniflora*, a typical estuarine wetland species, at different tidal flat elevations (low, middle and high) in filed and at different time (3, 6, 9, 12 h per day) of waterlogging treatment in greenhouse conditions. Results showed that the accumulation of copper, zinc, lead, and chromium in *S. alterniflora* was proportional to the exchangeable fraction of these metals in the sediments, which generally increased with the increase of waterlogging time, whereas the formations of iron plaque in roots decreased with the increase of waterlogging time. Under field conditions, the uptake of copper and zinc in the different parts of the plants generally increased with the tidal levels despite the decrease in the metals' exchangeable fraction with increasing tidal levels. The formation of iron plaque was found to be highest in the middle tidal positions and significantly lower in low and high tidal positions. Longer waterlogging time increased the metals' accumulation but decreased the formation of iron plaque in *S. alterniflora*. The binding of metal ions on iron plaque helped impede the uptake and accumulation of copper and chromium in *S. alterniflora*.

1. Introduction

Spartina alterniflora L., a perennial salt marsh plant native to Eastern North America, has been introduced to China in the 1990s to stabilize eroding coastal banks (Li et al., 2009; Smith and Lee, 2015). *S. alterniflora* (Alberts et al., 1990; Windham et al., 2001; Weis and Weis, 2004; Quan et al., 2007; Salla et al., 2011) and many other salt marsh plants, such as *Phragmites australias* Trin. (Windham et al., 2001; Quan et al., 2007; Weis and Weis, 2004; Altaisan, 2009), *S. maritima* (Reboreda and Caçador, 2008; Padinha et al., 2000), *S. densiflora* (Mateos-Naranjo et al., 2008, 2011; Idaszkin et al., 2015), and *S. argentinensis* (Redondo-Gómez et al., 2011), can accumulate large quantities of heavy metals (HMs), including copper (Cu), zinc (Zn), lead (Pb), chromium (Cr), manganese (Mn), nickel (Ni), cadmium (Cd), and arsenic (As), to the root with small amounts being transported to the aerial parts of the plants. Metals accumulated in the plants might also be released to the ambient environment due to the decomposition of plant litters or through the leaf salt excretion process of some species (Windham et al., 2001; Weis and Weis, 2004), and some of the HMs may enter the food web of the estuarine ecosystems (Dorgelo et al., 1995). Therefore, marsh plants can either be sources or sinks for HMs, which play important functions in biogeochemical cycling of HMs in estuarine wetlands (Weis and Weis, 2004; Chen et al., 2017).

The distribution and survival of salt marsh plants in coastal wetlands are determined mainly by the tidal inundation, and the frequency as well as duration of the tidal inundation vary greatly in the tidal flat (Huckle et al., 2000; Winkel et al., 2011; Brownstein et al., 2013; Duarte et al., 2014). The variation of the frequency and duration of the tidal flooding have profound influence on the adsorption and desorption behaviors of HMs in sediment through the modification of the redox potential (Eh), pH, dissolved organic carbon, redox chemistry of iron (Fe), Mn, and sulfur (S) (Zhu et al., 2012; Shaheen et al., 2014; Calvo-Cubero et al., 2016), and hence influence the accumulation and uptake of HMs in marsh plants (Thakur et al., 2016; Yan et al., 2017). Under waterlogging conditions, the Eh of the sediment usually decreases with increasing water inundation time, during which the insoluble Fe^{3+} and $Mn^{3+/4+}$ (hydr)oxides are reduced to soluble Fe^{2+} and Mn^{2+}, respectively, and the HMs that fixed to Fe^{3+} and $Mn^{3+/4+}$ (hydr)oxides are transformed into more mobile forms and released to soil pore water. Longer water inundation time may further decrease the Eh, and the sulfate in the sediment will be reduced to its metal-complexing form, sulfide (Reddy and DeLaune, 2008). With draining water,

* Correspondence to: State Key Laboratory of Estuarine and Coastal Researches, East China Normal University, 3663 Zhongshan Rd North, Shanghai, China.
E-mail address: zzyan@sklec.ecnu.edu.cn (Z. Yan).

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the sediments turned oxic, in which the co-precipitation or adsorption and subsequent immobilization of these metals occur. The extractable HMs in the sediments exert major influences on plants' growth and metal accumulation, and the fractionation of HMs in the sediments is closely correlated with the alternating hydrological regime of the sediment (Zhao et al., 2016).

To adapt to anaerobic conditions, wetland plants can produce aerenchyma, which allows the conduction of oxygen (O_2) from above ground plant parts to roots to maintain regular root respiration (Armstrong et al., 1992; Mainiero and Kazda, 2005). Iron (Fe) plaque is a common concomitant of radical oxygen loss (ROL) in the roots of wetland plants, which is induced by oxidation of Fe^{2+} to Fe oxides that are precipitated as plaque on the root surface (Armstrong, 1992; St-Cyr and Crowder, 1989). The effects of Fe plaque on metal uptake is controversial; conflicting results were reported as to whether the presence of the plaque reduces or increases the metal uptake of the plants (Ye et al., 1997; Batty et al., 2000; Tripathi et al., 2014). Most of the studies suggested that Fe plaque can bind with some toxic HMs and co-precipitate outside the root tissues, affecting the metal uptake in aquatic plants (Ye et al., 1997; Batty et al., 2000). Fe plaque was found to decrease the mobility of As, Mn, Cu, Pb, and Zn in some wetland plants, such as *Phragmites australis* (Batty et al., 2000), *Typha latifolia* (Blute et al., 2004) and *S. densiflora* (Cambrollé et al., 2008). Similar effects were also observed on Mn, Zn, Ni, Pb, Cr, and Cd in mangrove species, including *Bruguiera gymnorrhiza*, *Excoecaria agallocha, Acanthus ilicifolius*, and *Kandelia obovata* (Pi et al., 2011; Du et al., 2013).

The formation of Fe plaque in wetland plants are species-specific and are generally affected by various biotic and abiotic factors, such as oxidizing capacity of plant roots, texture, organic matter, and pH of the sediments (St-Cyr and Crowder, 1989; Armstrong et al., 1992; Tripathi et al., 2014). In China, hydrological regime (waterlogging or draining) of the coastal wetland sediments suffered great effects from reclamation activities (Ma et al., 2014) and extreme climate events (Duarte et al., 2015), which are both becoming more frequent because of the rapid economic development and climate change. To our knowledge, however, still very little is known about the Fe plaque formation in *S. alterniflora* and its roles in the HM accumulation and translocation in this plant under the influences from the periodic tidal inundation. The present study therefore aimed to investigate the effects of the periodic flooding and the variation of the physiochemical properties of the tidal flat on the uptake of HMs and formation of Fe plaque in *S. alterniflora*.

2. Materials and methods

2.1. Experimental setup

2.1.1. Field sampling

The frequency and duration of tidal inundation usually decrease as the elevation of the marsh increases (Huckle et al., 2000). In field conditions, *S. alterniflora* occupies a wide range of tidal levels with varied tidal elevations. Plants that grow in low tidal positions usually receive more waterlogging compared with those grown in high tidal positions. To investigate the variation of Fe plaque formation and HM accumulation in *S. alterniflora* at different waterlogging regimes under field conditions, we collected samples of the different plant parts of *S. alterniflora* and the ambient sediment in North Chongming Island on October 14, 2016 (Fig. S1 in the Supplementary Material). Three tidal positions (low, middle and high) were set along a transect with different distances from the tidal creek, in which the low tidal position was spaced 100 m apart from the tidal creek, the middle tidal creek spaced 200 m apart from the low tidal positions, and the high tidal position spaced 160 m apart from the high tidal positions. Triplicate samples (leaf, stem, and root) of *S. alterniflora* were collected at each tidal position, and the collected samples were placed into an ice chest and brought to the laboratory for HMs and Fe plaque measurements. During the sampling of plant samples, the Eh values of the rhizosphere sediments were measured in situ with an Oxidation Reduction Potential (ORP) meter (Spectrum IQ150, Spectrum Technologies Inc., USA), and the ambient sediment samples were also collected and brought to the laboratory for future measurement of soil texture, salinity, and total organic matter content.

2.1.2. Greenhouse-controlled experiment

To investigate the effects of different waterlogging times on the formation of Fe plaque and metal uptake in *S. alterniflora*, we designed automatic waterlogging systems to mimic the different tidal inundation times in real habitats. The waterlogging system was composed of an upper water tank [75 cm (L) × 60 cm (W) × 75 cm (H)], a polypropylene (PP) pot [50 cm (L) × 20 cm (W) × 40 cm (H)], and a lower water tank (with size the same as that of the upper water tank). PP pots were used for the cultivation of the seedlings, and holes with a diameter of 2 cm were punched evenly on the side walls of the pots for convenient water drainage. The PP pots sown with the seedlings of *S. alterniflora* were placed in the upper water tank. During the experiment, the treatment solutions in the lower water tank were pumped into the upper water tank that is automatically controlled by a time switch and kept in the upper water tank for a given period. Then, the solution in the upper tank was drained to the lower water tank through a time-controlled electric valve to complete a water cycle.

Seedlings of *S. alterniflora* with comparable size (approximately 40 cm in height) were collected in Nanhui, Shanghai. A total of 15 PP pots were prepared for the experiment. The collected *S. alterniflora* seedlings were carefully transplanted into the PP pots with the sediments collected from the ambient habitats. Each pot had six seedlings and contained approximately 6 kg of sediments. The physiochemical parameters of the sediments are summarized in Table S1 (Supplementary Material). The texture of the sediment was silt loam with TOM content of 8.8% ± 2.3%, and the background concentrations of Cu, Zn, Pb, and Cr are 12.5 ± 1.6, 62.2 ± 4.9, 5.8 ± 0.8, and 18.6 ± 1.0 mg Kg^{-1}, respectively. The pots were placed in greenhouse with a daily temperature of 20–30 °C, a relative humidity of 59–80%, and a light intensity of 800–1400 μmol photons $m^{-2} s^{-1}$. The seedlings were irrigated with tap water once daily until the start of the treatments. After 14 days of rejuvenation, seedlings were also randomly divided into five groups, and each group was set in triplicates. Four groups were placed into water tanks that received different waterlogging times (3, 6, 9, and 12 h) with treatment solution containing HMs, namely, 3 h + HMs, 6 h + HMs, 9 h + HMs, and 12 h + HMs. The pots were waterlogged twice a day to simulate the semidiurnal tide in Yangtze River estuarine. The treatment solution was prepared by dissolving appropriate amounts of $CuCl_2$, $ZnCl_2$, $PbCl_2$, and $CrCl_3$ into salt water (10‰ NaCl) to make the final concentrations of 23.4, 60.8, 18, and 11.2 mg L^{-1} for Cu^{2+}, Zn^{2+}, Pb^{2+}, and Cr^{3+}, respectively. The selection of metals and metal concentrations were based on a previous investigation on total bioavailable sediment HMs in Yangtze River estuarine (Chen et al., 2001).

Plant samples were collected 60 days after the treatment. One seedling in each pot was carefully pulled from the sediment and sufficiently washed with deionized water. Fresh lateral roots of the plants were collected and kept at 4 °C for the analysis of Fe plaque. The leaves, stems, and roots of the other plants were separated and dried in an oven at 70 °C for 48 h for the analyses of HMs. The sediments in the pots were also collected at the end of the experiments for the measurement of HMs. The collected sediment samples were air dried, grained in mortar, and then passed through a 1-mm sieve. The samples were stored in a desiccator for future analyses.

2.2. Determinations

2.2.1. Total and acid fraction HMs in sediments

For the measurement of total HMs in the sediments, 0.5 g of sediment sample was digested in a 50-mL polytetrafluoroethylene beaker with 5 mL of nitric acid, 1 mL of hydrofluoric acid, and 1 mL of perchloric acid. The residues of the sample digests were dissolved with 25 mL of 1% nitric acid. Cu and Zn in the digests of treatment solutions and sediments were analyzed by flame atomic absorption spectrometry (AAS, AAnalyst800, Germany). Pb and Cr were determined with an inductively coupled plasma optical emission spectrometer (ICP-OES, ICAP 7400, USA). Acid soluble fraction of HMs in the sediments was extracted according to the method by Ure et al. (1993). Briefly, an aliquot of approximately 0.5 g of sediment samples was transferred into a 50-mL polyethylene centrifuge tube, followed by addition of 30 mL of 0.1 mol L^{-1} acetic acid for extraction. The mixture was then sonicated for 16 h at ambient temperature. Subsequently, the mixture was centrifuged at 10,000 rpm for 20 min, the supernatant was collected, and the residues were washed two times with 10 mL deionized water, and centrifuged again. The supernatant was combined and made up to 50 mL in a volumetric flask. The HMs in the solutions were analyzed by AAS (PerkinElmer AAnalyst 800, USA) and ICP-OES (Thermo Scientific™ iCAP 7400, USA). Certified reference materials (CRM) of soils (GBW 07410, China National Center for Standard Materials) were used for the quality control. The average recovery and the standard error (SD) of Cu, Zn, Pb, and Cr in soil CRM were 96.3% ± 0.7%, 114.0% ± 8.0%, 87.1% ± 3.4%, and 83.5% ± 1.7%, respectively.

2.2.2. Physiochemical properties of the sediments

For measurement of sediment salinity, 8 g of the air-dried sediment samples were dissolved in deionized water in the ratio of 1:5 (w/v) and then filtered with filter paper. The electric conductivity of the filtrate was determined with an electric conductivity meter (Model CT-3031, Xi'an Yingheng Instrument Co., Ltd., China) and then converted into salinity (‰) by the empirical formula between electric conductivity and salinity. Total organic carbon (TOC) was estimated by the classic Loss-On-Ignition method (Heiri et al., 2001). In brief, the sediment samples were firstly oven dried at 105 °C overnight, cooled in a desiccator, and weighed before combusted at 550 °C for 6 h in a muffle furnace. After combustion, the samples were cooled in a desiccator and reweighed. The TOC content of the sediments was calculated using the following equation:

$$TOC\ \ (\%) = (W_{BC} - W_{AC})/W_{BC} \times 100,$$

where W_{BC} and W_{AC} are the sediment weights before and after combustion, respectively. The texture of the sediment (relative contents of clay, slit, and sand) and median diameter (MD) of the sediments were analyzed with a laser diffraction particle size analyzer (Model LS™ 13 320, Beckman Coulter Inc., USA). Sediments redox potential (Eh) were measured in situ by portable oxidation-reduction potentiometer (Model FJA-6, Nanjing Chuandi instrument Inc., China)

2.2.3. HMs in plants

Cd concentration in plant tissues was determined according to the method described by Wong et al. (1993). Approximately 0.2–0.3 g of oven-dried plant sample was humidified with 5 mL 1% HNO_3 and charred in a 10 mL crucible pot on a hot plate for approximately 1 h. During the charring of the samples, the temperature of the hot plate was controlled carefully to avoid the ebullition, and the crucible pot were always kept covered by a lid. The charred sample was then incinerated in a muffle furnace at 500 °C for 6 h. After incineration, the ash was digested and washed by 1% nitric acid, the lid of crucible pot were also washed triply with 1% nitric acid, and all the washings were combined and made up to 10 mL with 1% nitric acid. Cu, Zn, Pb, and Cr in the digested solutions were analyzed by AAS (PerkinElmer AAnalyst 800, USA). CRM of plants (GBW 10047) (China National Center for Standard Materials) were used for the quality control. The average recovery and the SD of Cu, Zn, Pb, and Cr were 127.5% ± 14.5%, 90.2% ± 5.4%, 98.2% ± 13.9%, and 88.7% ± 11.8%, respectively.

2.2.4. Fe plaque and heavy metals

Laterals of the plants were used for the measurement of Fe plaque content and HMs. The concentration of Fe plaque on root surface was determined by dithionite–citrate–bicarbonate (DCB) method according to Pi et al. (2011). Briefly, the collected root section was washed thrice with deionized water and then incubated in 15 mL of 0.3 mol L^{-1} sodium citrate ($Na_3C_6H_5O_7{\cdot}2H_2O$) and 2 mL of 0.125 mol L^{-1} sodium bicarbonate ($NaHCO_3$) with the addition of 1 g of sodium dithionite ($Na_2S_2O_4$) for 3 h at 25 °C. The extractant was collected, and the roots were washed thrice with 15 mL of deionized water. The washings were combined with the extractant and made up to 50 mL with deionized water in a volumetric flask. The concentrations of Cu, Cd, Pb, and Fe in DCB extractant were measured using ICP-OES. The washed roots were oven dried at 70 °C for more than 48 h to obtain the dry weight.

2.3. Statistical analyses

The mean and standard deviations of the three replicates for each treatment were calculated. Parametric one-way ANOVA and post-hoc multiple comparison (Tukey's test) were conducted to determine the significant differences in different parameters under different waterlogging time treatments and different tidal positions. The correlations between the soil physiochemical properties and the metal concentrations in different tidal flats were analyzed by Pearson correlation analysis. Principal component analysis (PCA) was used to determine the best linear combination of tidal sediments variables (including sediment TOM, texture, salinity, Eh, HM concentrations) that would account for the variance in the data as a whole than any other linear combination of variables. All the statistical analyses were performed with SPSS 16.0.

3. Results

3.1. Extractable and total heavy metal concentrations in different tidal positions

Along a transect from seaward to landward, the total concentrations of Cu and Zn generally increased from low to high tidal flat. The concentrations of Pb and Cr in the sediments that were collected from the low, middle, and high tidal levels were not significantly different (Fig. 1A). The acid fraction concentrations of Cu ($F = 1.30$, $p = 0.34$) and Zn ($F = 2.72$, $p = 0.11$)showed no significant changes along the transect, whereas the acid fraction concentrations of Pb and Cr decreased with the increasing tidal levels (Fig. 1B). We also calculated the ratios of extractable metal concentrations to the total metal concentrations, and the ratios of Cu and Zn decreased with increasing tidal levels, while the ratios of Pb ($F = 4.91$, $p = 0.06$)and Cr ($F = 3.43$, $p = 0.07$)showed no significant changes along the transect(Fig. 1C).

The clay content of the sediments in the middle and high tidal positions were significantly higher than that of the low tidal levels ($F = 22.89$, $p = 0.02$), whereas the silt content showed an opposite pattern (Figs. 2A and 2B). The median diameter (MD) of the sediments decreased significantly with the increase of tidal levels (Fig. 2C). Sediment salinity increased with the elevation of tidal levels, and the highest was observed at the middle tidal level (Fig. 2D). The TOM of the sediments increased with increasing tidal levels, but those of the sediments from the middle and high tidal levels were also not significantly different ($F = 10.41$, $p = 0.46$) (Fig. 2E). The Eh of the sediments increased significantly with the increase of tidal levels, with the lowest value (− 23.6 ± 2.4 mV) observed in low tidal levels and the highest value (130.1 ± 12.1 mV) in high tidal levels (Fig. 2F).

The TOM of the sediments was negatively correlated with the MD ($r = -0.837$, $p < 0.01$) and silt content ($r = -0.840$, $p < 0.01$) of the sediments and positively correlated with the clay content ($r = 0.830$, $p < 0.01$) of the sediments (Table S2 in the Supplementary Material). The MD of the sediment positively correlated with the silt

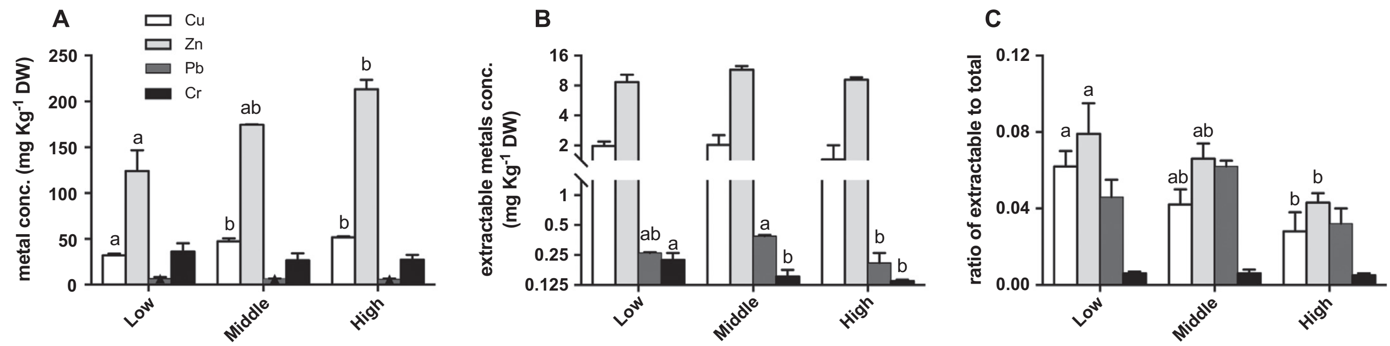

Fig. 1. Total and exchangeable HMs (Cu, Zn, Pb and Cr) in sediments at different tidal levels. (A) Total concentrations; (B) Exchangeable fraction concentrations; (C) ratios of exchangeable fraction to total HM concentrations (values are mean and SD; for each metal, data with different letters are significantly different at P ≤ 0.05; no letter appended if the data were not significantly different).

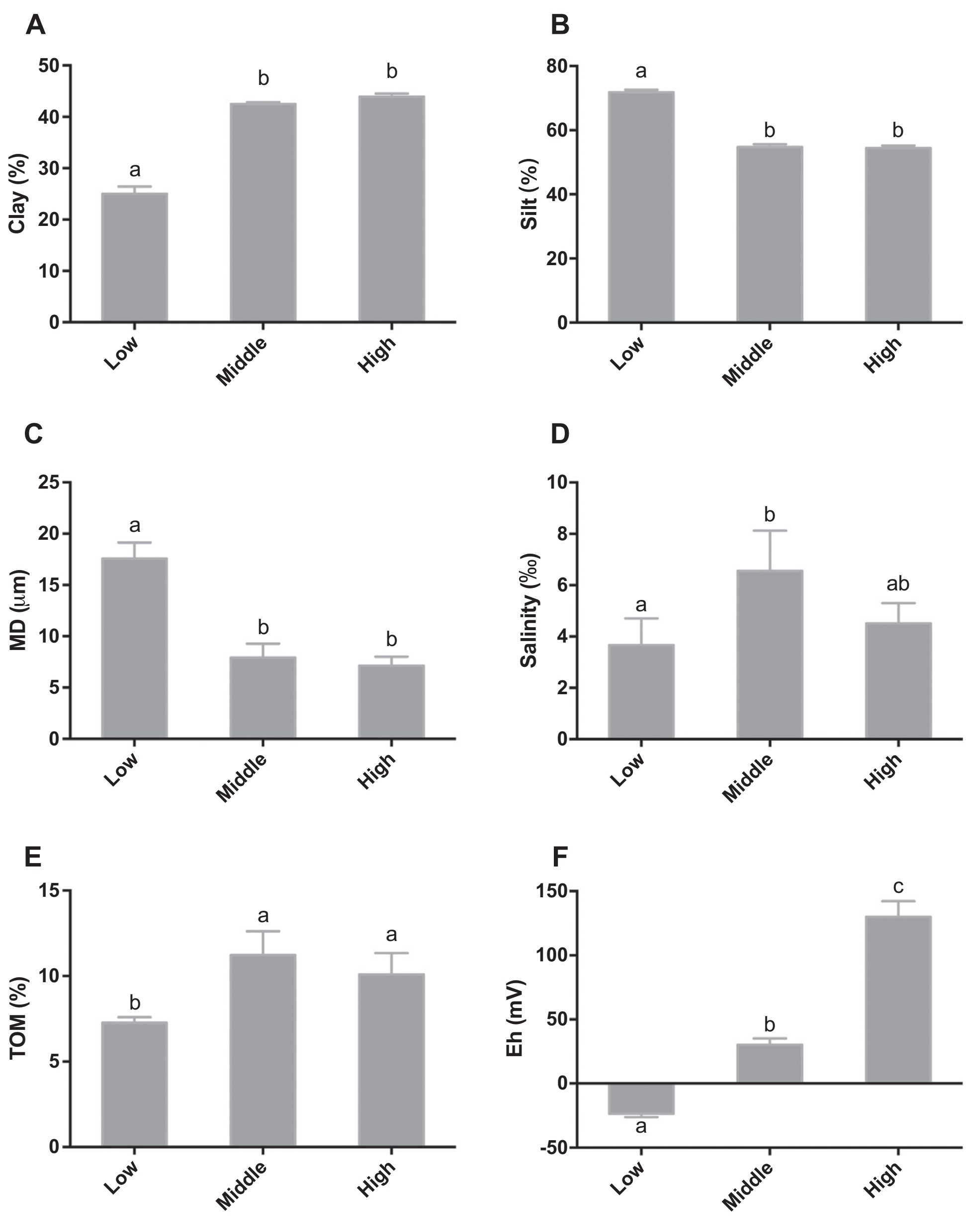

Fig. 2. Sediment physiochemical characteristics along different tidal levels. (A) clay content (%); (B) silt content; (C) MD (μm); (D) Salinity (‰); (E) TOM (%); (F) Eh (values are mean and SD; for each parameter, data with different letters are significantly different at P ≤ 0.05).

content (r = 0.996, $p < 0.01$) and negatively correlated with the clay content (r = −0.998, $p < 0.01$) and Eh (r = −0.779, $p < 0.05$) of the sediment. The Eh of the sediment negatively correlated with silt content (r = −0.770, $p < 0.05$) and positively correlated with the clay content (r = 0.802, $p < 0.01$). The silt and clay contents of the sediment were also negatively correlated (r = −0.997, $p < 0.01$). The concentrations of Cu in the sediments were found to be positively correlated with TOM (r = 0.712, $p < 0.05$), whereas the clay content and Eh of the sediments were negatively correlated with the MD and silt contents of the sediment. Similarly, Zn was also negatively correlated with the MD (r = −0.577, $p < 0.05$) and positively correlated with Eh (r = 0.564, $p < 0.05$) (Table S2 in the Supplementary Material). The concentrations of Pb and Cr in the sediments were positively correlated (r = 0.709, $p < 0.05$) with each other and both had significant negative correlations with Zn concentration (r = −0.683, $p < 0.05$; r = −0.670, $p < 0.05$) (Table S2 in the Supplementary Material).

According to the PCA analysis (Table S4 in the Supplementary Material), all the filed sediments physiochemical parameters (including HMs) can be represented by three principal components (PC), which in total explained 90.6% of the total variance. PC1 explained over 60% of the total variance, which was mainly characterized by the positive scores of TOM, clay, salinity, Eh, Cu and Zn contents, and Cu, Zn concentrations showed positive correlation with sediments TOM, clay contents and Eh according to Pearson correlation analysis. PC2 explained 19.7% of the total variance and was characterized mainly by the positive scores of salinity, Pb and Cr concentrations. PC3 only explained 10.7% of the total variance which was mainly characterized by positive scores of salinity and sand.

3.2. Metal accumulation in *S. alterniflora* that were collected from different tidal positions

Different plant parts of *S. alterniflora* were collected from different tidal positions, and the metal concentrations in the different plant parts are summarized in Table 1. The accumulation of Cu in the root and stem of *S. alterniflora* was found to be significantly higher in the high tidal position than that in the middle and low tidal positions(Table 1); however, accumulation of Cu in leaf did not vary significantly along different tidal positions. Accumulation of Zn in different plant parts was not statistically different at different tidal positions. The Pb and Cr concentrations in the root of *S. alterniflora* were found highest in the low tidal position, while their concentrations in the middle and high marshes were nearly equal. The accumulation of Pb and Cr) in the aboveground parts of *S. alterniflora* (i.e., leaf and stem) showed no significant difference among different tidal positions (Table 1).

3.3. Effect of different waterlogging times on metal accumulation in *S. alterniflora*

The Cu concentration in the root of *S. alterniflora* under different waterlogging times and HM treatments all increased significantly compared with that of the control group (F = 15.06, p = 0.001), while no significant differences were observed in the different combined treatments (i.e., 3 h + HMs, 6 h + HMs, 9 h + HMs, and 12 h + HMs). However, increasing waterlogging time significantly increased the Cu uptake in the leaves of *S. alterniflora* (Table 2). The changes of the concentration of Cu in the stems of *S. alterniflora* among different treatments were insignificant (F = 0.654, p = 0.637) (Table 2).

With the increase of waterlogging time, the accumulation or uptake of Zn in the different parts of *S. alterniflora* generally showed a dose-dependent increasing pattern, especially in the roots and leaves. The highest Zn accumulation was observed under the treatment of 9 h + 2HM and 12 h + HM, which is 2-fold of that measured under the treatment of 3 h + 2HMs (Table 2). The accumulation or uptake of Pb and Cr in the roots and leaves of *S. alterniflora* showed similar patterns with that of Zn, which both increased with increasing waterlogging time (Table 2).

The acid fraction concentrations of different HMs generally showed an increasing pattern with increasing waterlogging time (Table S3 in the Supplementary Material). The highest acid extractable Cu, Zn, and Cr were observed under the treatment with the longest waterlogging time (12 h + HMs). For Pb, 9 h + HMs had a significantly higher acid extractable concentration than that of the 3 h + HMs and 6 h + HMs treatments.

3.4. Fe plaque formation in the roots of *S. alterniflora* at different field tidal positions and under different waterlogging conditions

In the field, the Fe plaque concentrations in the roots of *S. alterniflora* were peaked in the middle tidal positions, while the lowest level of Fe was observed in the roots of *S. alterniflora* that were collected from the high tidal positions (Fig. 3). The concentrations of the metals, including Cu, Zn, and Cr, in the root DCB extractant all increased with the elevation of tidal levels, and the highest concentrations were all observed at the high tidal positions. However, the concentration of Pb in the root DCB extractant showed no significant difference among the different tidal positions (F = 0.414, p = 0.678) (Fig. 3). In the greenhouse-controlled waterlogging experiment, the highest Fe plaque concentration was found under the treatment of 3 h + HMs (Fig. 4). The concentrations of Cu and Cr in the DCB extractant were also highest under the treatment of 3 h + HMs, while the concentrations of Zn (F = 2.39, p = 0.12) and Pb (F = 2.33, p = 0.13) in the extractant showed no significant differences among different treatments (Fig. 4).

4. Discussion

The results of the present study showed that different metals showed different accumulation tendencies along different tidal positions. Cu and Zn generally increased with the elevation of tidal levels, whereas Cr showed an opposite trend, and Pb showed no significant variation. Increasing the tidal positions is usually accompanied by decreasing decrease in the inundation/waterlogging times and frequencies (Huckle et al., 2000), and the Eh of the sediments also increased significantly with increasing tidal levels according to the results of the present study (Fig. 2F). Higher redox potentials and more oxic nature of the high intertidal sediments may favor the accumulation of Fe^{3+} and $Mn^{4+/3+}$(hydrous) oxides, which were proven to have high adsorption affinity for HMs in sediments (Young, 2013). The increased Cu and Zn concentrations in the high intertidal sediments might be attributed to the adsorption of these metals on the insoluble Fe^{3+} and

Table 1
Accumulations of Cu, Pb, Zn and Cr in different parts of *S. alterniflora* at different tidal position (Data of each plant parts with different letters indicate they are significantly different at the level of P ≤ 0.05, no letter appended if the data is not significantly different).

Tidal position	Cu (μg g^{-1} DW)			Zn (μg g^{-1} DW)			Pb (μg g^{-1} DW)			Cr (μg g^{-1} DW)		
	leaf	stem	root	leaf	stem	root	leaf	stem	root	leaf	stem	root
Low	8.5 ± 0.7	6.7 ± 0.2^{a}	14.7 ± 2.9^{a}	27.2 ± 3.7	41.1 ± 17.9	12.1 ± 7.9	0.7 ± 0.3	1.7 ± 0.7	3.0 ± 0.5^{a}	1.1 ± 0.0	4.0 ± 0.1	10.9 ± 0.8^{a}
Middle	7.2 ± 0.3	6.1 ± 0.4^{a}	17.3 ± 1.9^{a}	20.3 ± 3.5	36.5 ± 10.5	23.0 ± 4.8	0.5 ± 0.3	1.0 ± 0.2	1.4 ± 0.0^{ab}	0.5 ± 0.2	2.4 ± 1.2	7.5 ± 1.5^{b}
High	9.3 ± 1.3	9.9 ± 0.2^{b}	25.8 ± 0.4^{b}	22.7 ± 3.1	57.1 ± 17.8	30.7 ± 13.5	0.5 ± 0.1	1.0 ± 0.1	1.2 ± 0.2^{b}	0.7 ± 0.1	2.5 ± 0.9	6.7 ± 1.9^{b}

Table 2
Accumulations of Cu, Zn, Pb and Cr in different plant parts 60 days after different time of waterlogging treatments (metals concentration in each plant parts with different letters indicate they are significantly different at the level of $P \le 0.05$, no letter appended if the data is not significantly different).

Heavy metals conc. ($\mu g \cdot g^{-1}$DW)	Plant part	6 h	3 h + 2HM	6 h + 2HM	9 h + 2HM	12 h + 2HM
Cu	Root	15.76 ± 1.46^{b}	91.89 ± 20.51^{a}	78.64 ± 17.91^{a}	84.64 ± 14.24^{a}	102.61 ± 14.66^{a}
	Stem	15.62 ± 3.60	17.88 ± 2.98	22.03 ± 5.65	20.87 ± 9.17	17.73 ± 4.02
	Leaf	8.13 ± 1.24^{a}	8.36 ± 0.19^{a}	18.75 ± 6.01^{b}	13.45 ± 3.78^{ab}	18.92 ± 6.40^{b}
Zn	Root	21.95 ± 8.74^{d}	91.81 ± 19.62^{b}	163.16 ± 8.74^{c}	248.87 ± 7.77^{a}	230.41 ± 8.74^{a}
	Stem	34.20 ± 2.15^{c}	86.74 ± 19.50^{abc}	86.02 ± 15.15^{bc}	144.08 ± 29.09^{a}	115.00 ± 29.31^{ab}
	Leaf	26.50 ± 5.70^{a}	70.96 ± 23.96^{a}	148.59 ± 21.39^{b}	142.20 ± 22.78^{b}	138.51 ± 7.01^{b}
Pb	Root	1.39 ± 0.04^{e}	1.99 ± 0.54^{c}	3.76 ± 0.27^{d}	5.55 ± 0.27^{b}	7.82 ± 0.09^{a}
	Stem	0.77 ± 0.23^{c}	0.87 ± 0.45^{bc}	1.08 ± 0.12^{bc}	2.34 ± 0.75^{a}	1.89 ± 0.08^{ab}
	Leaf	0.31 ± 0.02^{b}	0.62 ± 0.28^{b}	0.90 ± 0.19^{ab}	1.64 ± 0.59^{a}	1.66 ± 0.11^{a}
Cr	Root	2.00 ± 0.97^{c}	4.63 ± 0.65^{bc}	8.03 ± 2.99^{ab}	4.48 ± 0.13^{bc}	8.76 ± 0.13^{a}
	Stem	0.77 ± 0.27^{a}	1.19 ± 0.30^{ab}	2.01 ± 0.71^{b}	1.36 ± 0.14^{ab}	2.19 ± 0.54^{b}
	Leaf	1.57 ± 0.58^{a}	2.51 ± 0.50^{ab}	3.49 ± 0.28^{ab}	4.80 ± 1.85^{b}	5.01 ± 1.75^{b}

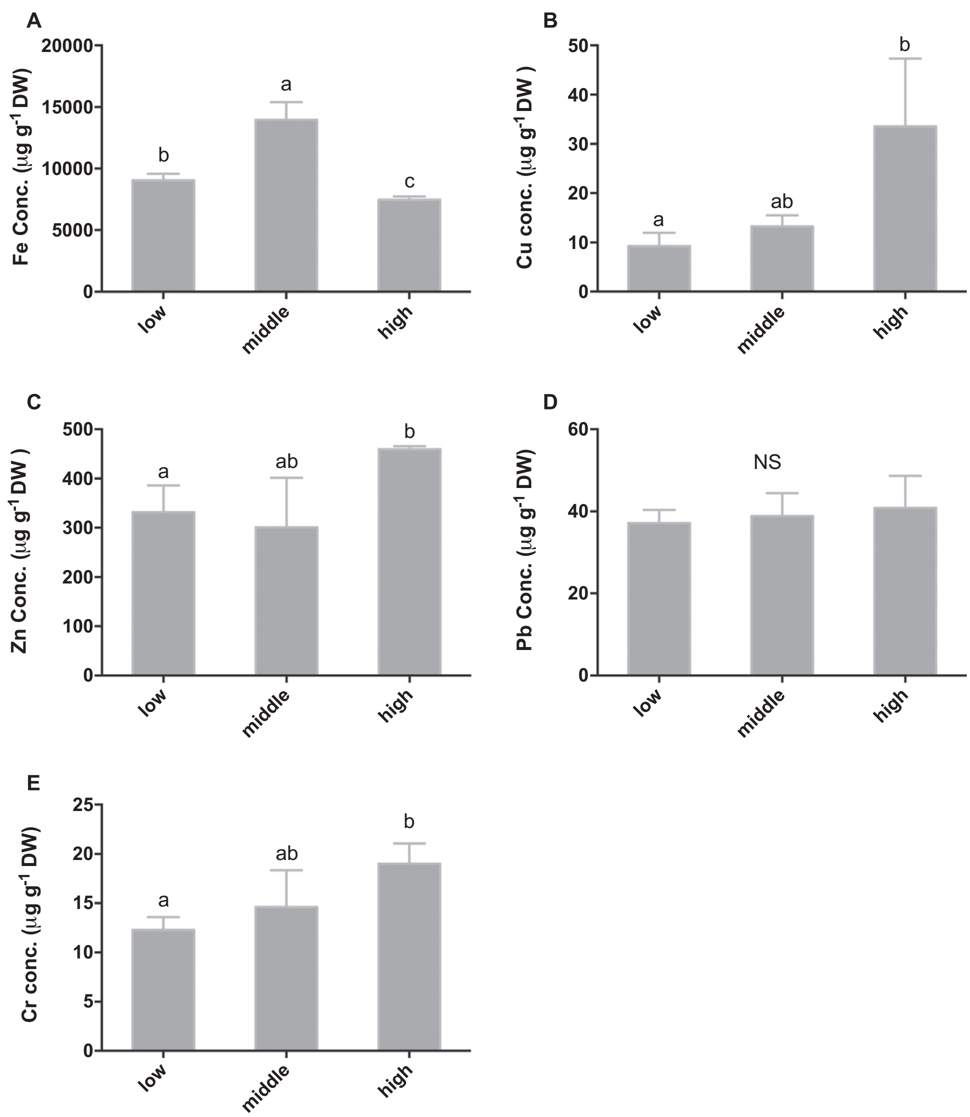

Fig. 3. Concentration of (A) Fe, (B) Cu, (C) Zn, (D) Pb and (E) Cr in DCB extractant of S. alterniflora grows at different tidal levels (values are mean and SD; for each parameter, data with different letters are significantly different at $P \le 0.05$; NS: not significant).

$Mn^{4+/3+}$ hydrous oxides. In comparison, the more reduced environment in the front bare flat and low marshes favors the reduction of Fe^{3+} and $Mn^{4+/3+}$ to more soluble Fe^{2+} and Mn^{2+}, which release to the pore water associated with the HMs and were flushed away with the tidal waters and may thereby decrease the accumulation of the metals in the low marsh sediments.

With increasing tidal levels, the MD of the sediments decreased significantly, whereas the TOC increased significantly (Fig. 2). Person correlation analysis also showed that both Cu and Zn concentrations were negatively correlated with the MD, whereas the Cu concentrations were, positively correlated with the TOC in the sediment. Our experimental results demonstrate that the grain size distribution of sediment exerts important effects on the concentrations and bioavailability of HMs in sediments and highest concentrations of metals are normally

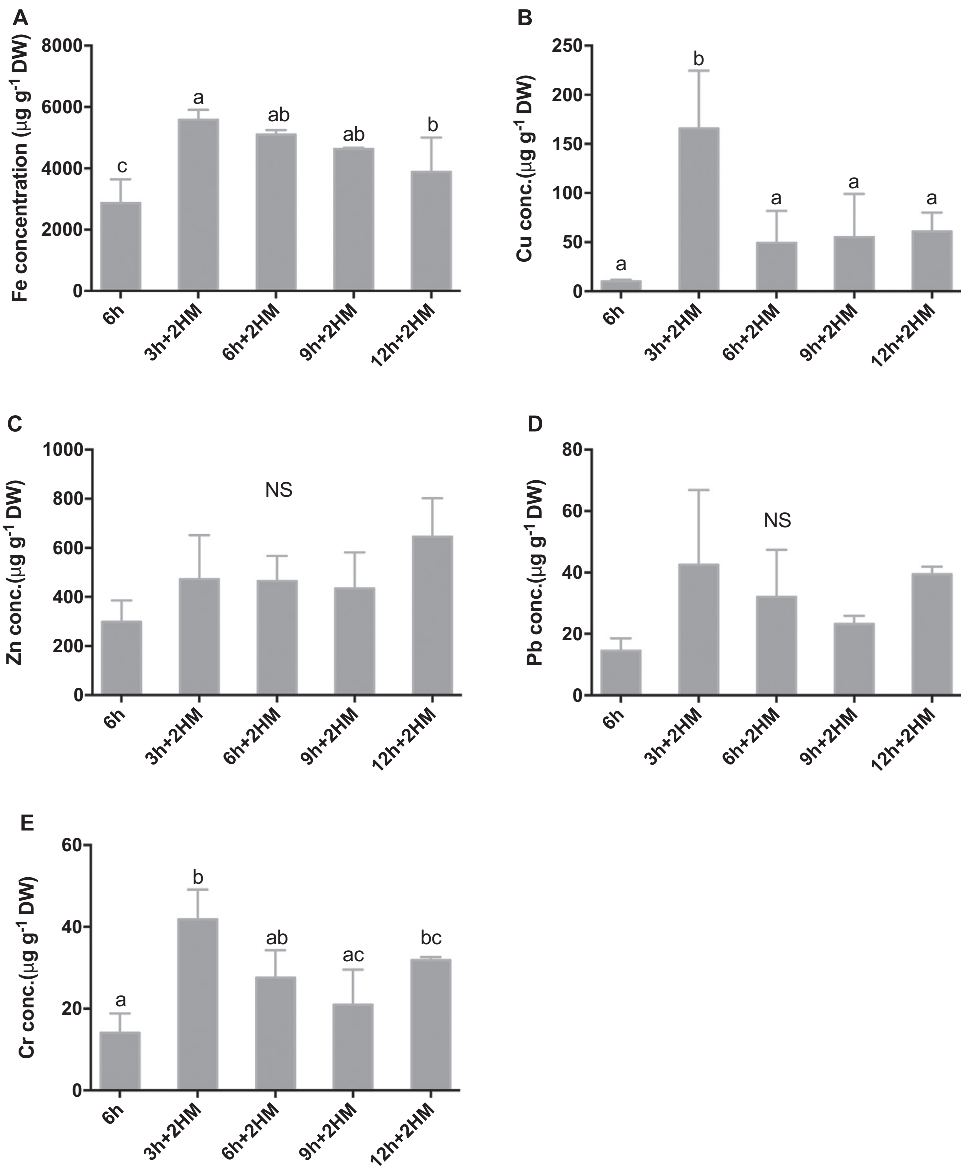

Fig. 4. Concentration of (A) Fe, (B) Cu, (C) Zn, (D) Pb and (E) Cr in DCB extractant of *S. alterniflora* under treatment with different time of waterlogging and HMs (values are mean and SD; for each parameter, data with different letters are significantly different at $P \le 0.05$; NS: not significant).

associated with fine-grained sediment particles, which agree well with previous studies (Chakraborty et al., 2015a). This finding may be attributed to the fact that small-sized clay–silt particles usually have larger surface areas, which increase the association of HMs with these particles. Heavy elements are also bound to various insoluble organic forms, such as living organisms, detritus, and humic material (Prasad, 2004). The higher organic matter content in the high marsh sediments also favored the accumulation of HMs. The present study also shows that the ratios of the exchangeable fraction of the Cu and Zn in the sediment decreased significantly with increasing tidal levels, which might be attributed to the fact that the sediments of the high tidal levels were more oxic (indicated by the relatively higher Eh value according to Fig. 2F) than that of the low tidal levels, favoring the formation of Fe^{3+} and $Mn^{4+/3+}$(hydrous) oxides, which bind more Cu and Zn, and therefore, decreases the exchangeable fraction. In the controlled waterlogging experiments, we also found a similar pattern, in which the exchangeable fractions of Cu, Zn, Pb, and Cr were generally lower in the treatment that had shorter waterlogging time. The PCA analysis also showed that Cu, Zn concentrations, TOM and clay contents as well as the sediment Eh all had high positive scores on PC1, while Pb, Cr concentrations and sediment salinity had high positive scores on PC2, suggested the variations of sediments major physiochemical parameters (including TOM, Eh and clay) had strong linear correlations with the accumulation of Cu and Zn in sediments.

Desorption and adsorption behaviors of Cr in the sediments are more complicated than those of the other three metals. On the one hand, Cr in the soil may also adsorb on the Fe^{3+} and $Mn^{4+/3+}$ hydrous oxides when Eh is high and released to the pore water when the Eh declined. On the other hand, Cr in the sediments normally has two valence states: the soluble Cr^{6+} and the insoluble Cr^{3+}. With decreasing sediment Eh, more Fe^{2+} are generated, thereby favoring the reduction of soluble Cr^{6+} to insoluble Cr^{3+} (Stanin et al., 2004). In the present study, the highest Cr concentrations were found in the bare flat sediments and increased with the tidal levels despite the fact that the differences among the low, middle, and high marshes were not statistically different. The more reduced sediment condition in the bare flat and low tidal levels likely favored the accumulation of the insoluble Cr^{3+}, thereby increasing its concentrations in the sediments.

The mobility and bioavailability of HMs are dependent on their specific chemical forms in soils rather than in the total elemental contents (Zhu et al., 2015; Lutts and Lefèvre, 2015). HMs in exchangeable and carbonate forms are easier to be taken up by plants than the other forms (Chakraborty et al., 2015b). The metal uptake is usually proportionally related to the metal concentrations in the external medium

(Prasad, 2004). In the controlled waterlogging experiments of the present study, the accumulation of different metals (Cu, Zn, Pb, and Cr) in different plant parts generally increased with the increase of waterlogging time. This changing pattern is in accordance to the changes of the exchangeable fraction of these metals in the sediments along the different waterlogging treatments (Table S3 in the Supplementary Material). These results suggested that the accumulation or uptake of these metals are strongly controlled by their exchangeable fractions in the sediments. The uptake and accumulation of HMs in *S. alterniflora* under the intertidal environments are more complicated than that under the controlled waterlogging experiments. Under field conditions, the accumulations of different metals in plant parts along the different tidal levels were metal specific. The uptake and accumulation of Cu and Zn in the different parts of the plants generally increased with the tidal levels despite decreasing metal exchangeable fraction with increasing tidal levels. The accumulation of Cu, Zn, Pb, and Cr in the different plant parts seemed to be associated with the total concentration of these metals in the sediments, as they changed similarly along different tidal levels. The uptake of HMs in aquatic plants usually varied with the metal concentration and physiochemical properties of the sediments, such as the content of organic matters, pH, salinity, and Eh (Thakur et al., 2016). In the intertidal environments, the physiochemical factors that influence the uptake and accumulation of HMs in the plants all varied greatly at different tidal positions, while for the indoor controlled waterlogging experiments, the major influencing factor is the waterlogging time. The organic matter content of the sediments has been suggested to increase the metal availability and metal uptake in the plant roots, and the bioavailability of the metals are normally negatively correlated to the sediment pH values (Prasad, 2004; Du Laing et al., 2009; Tőzsér et al., 2017). Previous study also suggested that the sediment pH increases with the increase of flooding time (Kashem and Singh, 2001). In the present study, the sediments of the high tidal level received relatively shorter flooding time, and their pH values were likely lower than that of the low tidal levels. The TOC contents of the high tidal levels were also higher than those of the sediments at low tidal levels. The relatively low pH and high TOC content of the sediments in the high tidal levels both increased the bioavailability of HMs in the sediment and favored the uptake of HMs in the plant.

The formation of Fe plaque in the roots of wetland plants can be influenced by either abiotic or biotic factors, such as the aeration conditions of the sediments and the oxidizing capacity of the plant roots (Armstrong, 1979; St-Cyr and Crowder, 1989; Batty et al., 2000; Cheng et al., 2010; Wu et al., 2012). The radial oxygen loss (ROL) of roots, as part of the biotic factors, was suggested to play vital functions in Fe plaque formation in wetland plants, and higher ROL induces more Fe plaque formation on the root surface (Armstrong et al., 1992). In the present study, *S. alterniflora*, which received short time and low frequency of waterlogging (3 h + HMs), had the highest Fe plaque formation in the root surface, and the formations of Fe plaque generally decreased with increasing waterlogging time. This occurrence was probably attributed to the facts that the rhizosphere of the plants under waterlogging treatment at short times is more oxic and that the ROL rates of the roots were higher than that under waterlogging treatments at longer times, which both favored the formation of Fe plaque on the root surface. Similar results were also observed in rice, in which the external aeration treatment significantly increased the Fe plaque formation in rice roots than in those treated with stagnant solution (Wu et al., 2012). Normally, the root ROL of wetland plants can be enhanced when plants are subjected to waterlogging (Van Bodegom et al., 2005). However, roots did not show a continued increase in their ROL rates indefinitely as the external demand increases (Sorrell, 1999) because the ROL rates of wetland plants are controlled by the oxygen demand in the rhizosphere, such as root respiration and respiratory activities of microorganisms at low to moderate reduction intensities (Van Bodegom et al., 2005). This phenomenon explains why the Fe plaque formation under the longest waterlogging treatments (12 h + HMs) were significantly reduced compared with that under the relatively short waterlogging treatments (3 h + HMs, 6 h + HMs and 9 h + HMs). Moreover, some previous studies also showed that HMs in the sediments might also decrease the permeability and ROL in the roots of some mangrove plants. HMs (Pb, Zn, and Cu) have been found to decrease ROL in the roots of *Aegiceras corniculata*, *Avicennia marina*, and *Bruguiera gymnorrhiza* seedlings and change the spatial pattern of ROL (Liu et al., 2009). In the present study, the sediments with longest waterlogging treatment (12 h + HMs) had significantly higher acid fraction concentration of HMs than those that received shorter waterlogging treatment (Table S3 in the Supplementary Material). The relatively higher HM contents in the sediments with longer waterlogging times might also diminish Fe plaque formation in *S. alterniflora* under the 12 h + HMs treatment.

In field conditions, the formation of Fe plaque was found to be highest in the middle tidal positions and significantly lower in low and high tidal positions. These results were somehow different compared with those observed in the controlled waterlogging experiments because the aeration condition of the sediments in the high tidal levels ought to be higher or at least equal to the sediments under the middle and low tidal levels, which might favor the formation of more Fe plaque on the roots of *S. alterniflora*. Notably, the sediments in the field conditions is considerably more complicated than that in the controlled greenhouse conditions because the sediments' physiochemical properties, including texture, TOC, and salinity, all varied greatly across the different tidal levels. According to previous studies, the formation of the aerenchyma tissues, which is the basis for the ROL in the root, is negatively correlated with nutrient availability in soil (Videmšek et al., 2006). In the present study, the TOC increased significantly with the tidal levels, and the increased nutrient availability in the high tidal position might limit the production of aerenchyma tissues in the roots and more living cells in the roots might favor internal oxygen consumption and decrease the ROL of the roots; therefore, the Fe plaque formation in roots at high tidal positions were depressed.

The Fe plaque can bind with some toxic HMs and co-precipitate outside the root tissues, affecting the metal uptake of aquatic plants (Ye et al., 1997; Batty et al., 2000). In the controlled waterlogging experiment of the present study, waterlogging at shorter time induced more Fe plaque formation on the root surface of *S. alterniflora*, HMs (especially Cu and Cr) in the DCB extractant of the roots that received shorter waterlogging treatment are also higher than those that received longer waterlogging. These results suggested that the Fe plaque plays some roles in binding some metal ions in the roots of *S. alterniflora*. Similar results were also observed under field conditions; the concentrations of HMs (Cu, Zn, and Cr) in the DCB extractant were higher in the plant roots collected from the high tidal positions. The binding of metal ions on Fe plaque likely helped impede the metal uptake and accumulation in *S. alterniflora* because the metal (Cu, Zn, Pb, and Cr) concentrations in the roots and leaves of *S. alterniflora* all increased with the increase of waterlogging time, which were averse to the changes of Fe plaque formation along the waterlogging treatments. These results agree with most of the previous studies that demonstrate that the formation of Fe plaque in the roots of wetland plants may help prevent the uptake of toxic metals in root tissues (Batty et al., 2000; Pi et al., 2011; Du et al., 2013). Nevertheless, Fe plaque has been suggested to be of little significance in the limited uptake of HMs in the mangrove plant, *K. obovata*, and the adsorption of HMs in the root surface is the major barrier that impedes the uptake of HMs (Du et al., 2013).

5. Conclusion

The oxic/anoxic nature and grain size distribution of the sediments exert great effects on the accumulation of HMs, and the Cu and Zn concentrations in the sediments at different tidal levels were positively correlated with sediment Eh and negatively correlated with sediment MD and silt content. The accumulation of HMs (Cu, Zn, Pb, and Cr) in the different plant parts generally increased with the increase of waterlogging time and was proportionally related to the exchangeable fraction of these metals in the sediments. These findings suggest that the accumulation or uptake of these metals are strongly controlled by their exchangeable fraction in the sediments. The formation of Fe plaque in the roots of *S. alterniflora* generally decreased with the increase of waterlogging time. More Fe plaque were formed with significantly higher Cu and Cr concentrations in the DCB extractant in the roots of *S. alterniflora* that received short waterlogging treatment, suggesting that the Fe plaque formation in *S. alterniflora* was influenced by the aerobic condition of the sediments and can selectively bind with some HMs, such as Cu and Cr. The binding of HMs in the Fe plaque possibly play a role, at least in part, in the limited accumulation/uptake of HMs in *S. alterniflora* because the concentration of the HMs in the different parts of *S. alterniflora* that received short waterlogging treatment was significantly lower than those that received longer waterlogging treatment with the addition of same strength of exogenous HMs. Under field conditions, the accumulation of HMs and the formation of Fe plaque in *S. alterniflora* were different from that in the controlled waterlogging experiment. Such result might be associated with the comprehensive function of waterlogging and other physiochemical properties of the sediments under the field conditions, such as the variations in texture, TOM, salinity, and Eh.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.ecoenv.2018.02.008.

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