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THEMATIC ISSUE

Heavy metal distribution and accumulation in the *Spartina alterniflora* from the Andong tidal flat, Hangzhou Bay, China

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Abstract In order to study the heavy metal accumulation and distribution in the roots, stems, and leaves of *Spartina alterniflora*, we collected *S. alterniflora* samples and the associated sediments along three transects at the Andong tidal flat, Hangzhou Bay. Co, Ni, Cd, Pb, Cu, and Zn were mainly accumulated in the aerial parts (stems and leaves) of the plants, and their distributions depended on their mobility and their roles during the metabolism processes of *S. alterniflora*. The concentrations of Cu, Zn, Cd, Hg, and Pb were significantly enhanced with the increasing of heavy metal concentrations in the sediments, while those of Co and Ni remained relatively constant. Bioaccumulation factors results showed that the serious heavy metal contamination in the sediments from the transect A may overwhelm the accumulation capability of the plants. In addition, the physicochemical properties of the sediments and the pore water therein also play a role in the heavy metal concentrations and accumulations in the plants, because they can influence the behaviors and bioavailabilities of heavy metals during nutrition and bioaccumulation processes of the plants. The sediments with vegetation did not show significantly decreased heavy metal concentration with respect to the unvegetated sediments, although the plants did absorb heavy metals from the sediments. Principal component analysis and correlation analyses indicated that Co–Ni, Cu–Cd–Hg behaved coherently during accumulation, which may be ascribed to their similar accumulation mechanisms. This work provided essential information on the heavy metal accumulation by plants in a tidal flat, which will be useful for the environmental control through phytoremediation at estuaries.

Keywords Heavy metals · Accumulation · *Spartina alterniflora* · Sediment · Estuary · Hangzhou Bay

Introduction

Heavy metals are harmful and widespread in the environment, especially in the estuary zone such as tidal flats and salt marshes. They may originate from both natural and anthropogenic processes (Markert and Friese 1999). These pollutants are characterized by high toxicity, persistence, and bioaccumulative behaviors. Previous studies suggested that heavy metals may pose a significant threat to human health and living organisms and therefore produce a serious damage for natural ecosystem (Bryan and Langston 1992; Williams et al. 1994; Wong et al. 2002; Diagomanolin et al. 2004; DeForest et al. 2007). Consequently, it is important to study the heavy metal pollution within the studied coastal environment (Marcovecchio 2000; Hempel et al. 2008).

Numerous studies reported the occurrence and distribution of heavy metals in the sediments and plants from tidal flats at estuary zones (Marcovecchio 2000; Ferrer et al.

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2006). The periodical tide can carry large quantities of pollutants which can be accumulated in the sediments of tidal flats. In addition, the tidal flat plays an important role in the biogeochemical cycling of pollutants through their active and positive circulation mechanisms (Weis and Weis 2002). Vegetation can absorb the nutrients and metals from the sediments when their concentrations are relatively high (Reboreda and Cacador 2007a; Cacador et al. 2009; Almeida et al. 2004; Weis et al. 2003; Windham et al. 2003). The bioaccumulation process is depended on the mobility and bioavailability of metals as well as the physicochemical characteristics of the sediments such as pH, salinity, redox potential, organic matter content, grain size (Alloway et al. 1990). These physicochemical properties could change the microenvironment and therefore affect the bioavailability of metals in the tidal flats (Windham and Lathrop 1999; Windham et al. 2003). The uptake and accumulation of heavy metals by plants follow two different paths: (1) by the root system and (2) by the foliar surface (Sawidis et al. 2001). Path (1) is usually the dominated process. Therefore, tidal flat is often considered to be a sink for heavy metals (Cacador et al. 1996, 2000; Weis et al. 2004; Reboreda and Cacador 2007b), where the heavy metals play key roles for the local ecological systems.

Hangzhou bay is a typical macro-tidal estuary along the east coast of China, and the Andong tidal flat is one turning point of the hydrodynamic environment within Hangzhou Bay. It is widely accepted that the Andong tidal flat is the transition zone from middle tidal to spring tidal, open sea to semi-enclosed sea, and also the influence boundary between the Qiantang River and Yangtze River (Su and Wang 1989). In recent years, Hangzhou Bay suffered high urbanization and industrial activities. Large quantities of pollutants were discharged into Hangzhou Bay (Marcovecchio and Ferrer 2005; McGann 2008). Therefore, the heavy metal contamination in Hangzhou Bay attracted great attentions (Zhang et al. 2001; Deng et al. 2004). For instance, Liu et al. (2012) investigated the distribution of major and trace elements in the surface sediments of Hangzhou Bay and suggested that the anthropogenic impact was enriched near the Qiantang River mouth.

The Andong tidal flat in Hangzhou Bay is largely covered by *Spartina alterniflora*. In China, *S. alterniflora* was intentionally introduced from North America in 1979 to control the erosion of flats, improve the soil quality and protect the dikes. Nowadays, *S. alterniflora* has been a predominant macrophytes in the estuaries of China (Huang et al. 2008; Jiang et al. 2009; Zuo et al. 2009). *S. alterniflora* can accumulate metals from sediments via roots and transport some of them to its aerial structures. The metals can be stored in the belowground parts as well (Weis and Weis 2004). In addition, *S. alterniflora* can transport oxygen through aerenchyma to the rhizosphere, and therefore the absorbed metals are enriched in the roots (Burke et al. 2000). This phenomenon is similar to the cases of other marsh plants that the absorbed metals tend to accumulate in the belowground tissues (Burke et al. 2000; Reboreda and Cacador 2007a; Hempel et al. 2008; Duarte et al. 2010). Current studies on the *S. alterniflora* at coastal zones of China mainly focused on its ecological and physiological characteristics (Gallagher et al. 1980; Fang et al. 2004; Davis et al. 2004), its restoration for tidal flats (Gallagher et al. 1976; Gallagher and Plumley 1979; Valery et al. 2004; Mendelssohn and Kuhn 2003), its effect on the biogeochemical processes of intertidal ecosystem in coastal region (Zhou et al. 2008), and its influence on the uptake and distribution of N, P, and metals in the Yangtze River estuary (Quan et al. 2007).

To date, however, the research on the accumulation and distribution of heavy metals in the organs of *S. alterniflora* within Hangzhou Bay is scarce. The main objectives of this study are to: (1) assess the accumulation and distribution of heavy metals (Co, Ni, Cd, Hg, Pb, Cu, and Zn) in the organs of *S. alterniflora* and the associated sediments from the Andong tidal flat; (2) comprehend the relationship between heavy metal concentrations in the plants and sediments and explore the role of *S. alterniflora* on the translocation of heavy metals in the ecosystem of tidal flat; (3) preliminarily identify the accumulation mechanisms of heavy metals by the *S. alterniflora*.

Geological settings

Hangzhou Bay, which lies in the northeast of Zhejiang province and covers an area of about 8500 km^2, embodies one of the largest tidal gulfs in the world (Xie et al. 2010). Hangzhou Bay can be divided into an inner bay and an outer bay according to the hydrological and sediment characteristics of the bay (Fig. 1) (Chen et al. 1990). The sediments of the triangle shaped Hangzhou Bay mainly come from: (1) the particles carried by the Qiantang River and Yangtze River and forced by the river flows and tidal currents (Milliman et al. 1985; Su and Wang 1989); (2) sediments formed by the erosion of the seabed of the East China Sea (Dai et al. 2014). The sediments from the Yangtze River and Qiantang River played an important role in the distribution of heavy metals in the sediments and the anthropogenic influences were concentrated near the Qiantang River mouth (Li and Xie 1993; Liu et al. 2012; Pan and Wang 2012).

The Andong tidal flat, which is located at the southern part of Hangzhou Bay, situates near the boundary between the inner bay and outer bay. The Andong tidal flat is composed by tidal flats, tidal slopes, and tidal creeks, covering an area of about 300 km^2. The tidal flat is dominated by silt and clayed silt (Li and Xie 1993). The clayed

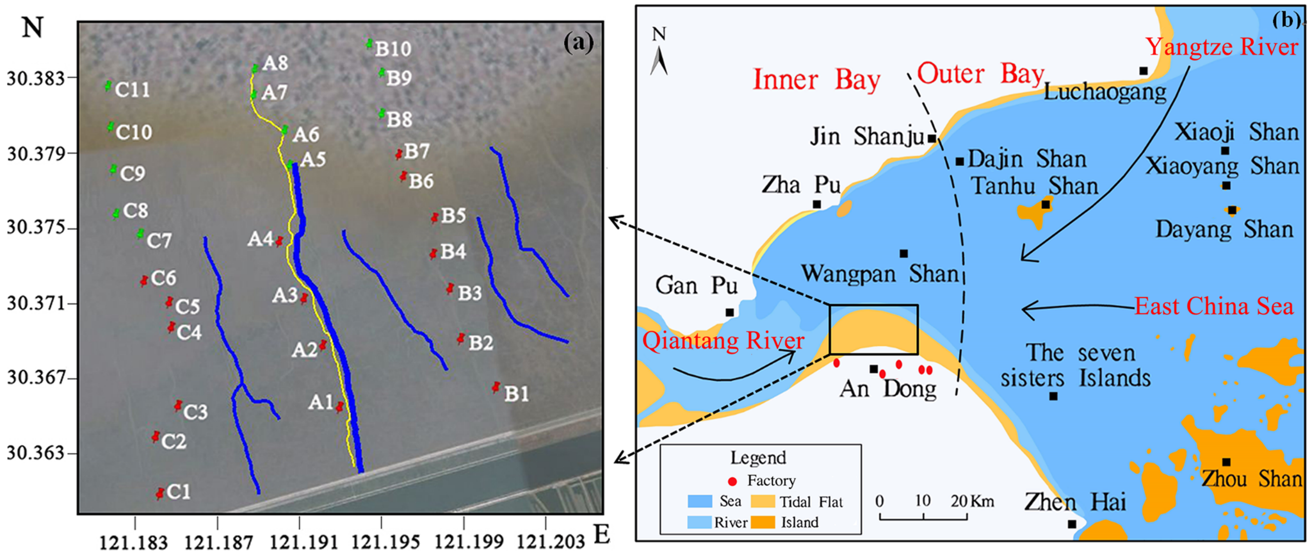

Fig. 1 **a** Detailed sampling sites and **b** the geographic location of the studied area in Andong tidal flat, Hangzhou Bay, China. The blue lines indicate the main tidal creeks along the sampling transects. The yellow line shows the passageway of local fishermen to catch aquatic products. The red tags indicate the plant samples and the associated sediments samples, while the green tags are the unvegetated sediment samples. The red spheres in (**b**) indicate the factories near the Andong tidal flat, including chemical plants, steel plants, plastics factories

silts are mainly distributed in the nearshore areas, and the silt sediments are widespread in the tidal flat and tidal creek areas. The sedimentation rate of the Andong tidal flat was 2.0–4.5 cm a^{-1} in recent centuries (Li 1993; Guo et al. 2004), and the average annual deposition volume of sediments was 6×10^7 t a^{-1} in recent decades (Li and Xie 1993). The Andong tidal flat received considerable anthropogenic pressures from engineering constructions, local aquaculture, and industrial activities from adjacent towns and cities (red spheres in Fig. 1b).

The Andong tidal flat is mainly covered by halophyte vegetation with a width of about 5 km from shore to offshore and an area of 100–150 km^2. *S. alterniflora* is the most commonly and widely distributed speciation. Our previous study investigated the heavy metal concentrations in the sediments from the Andong tidal flat and found that a transect was severely polluted by heavy metals (Pang et al. 2015). Therefore, it is essential to investigate the accumulation and distribution of heavy metals in the *S. alterniflora* from the Andong tidal flat, which may provide important information for the ecological, environmental, and geochemical studies on estuaries.

Materials and methods

Sampling

We collected 17 plant samples and 29 associated sediment samples from the Andong tidal flat during low tide. All of our samples were collected by hands that wore pre-cleaned gloves. We conducted three transects in this tidal flat, and the sample locations were recorded by a handhold GPS (Fig. 1). The samples from the transect A (A1–A8) were collected on August 14, 2014; the samples from the transect B (B1–B10) and transect C (C1–C11) were collected on September 14, 2014. Both sampling days were cloudy with light raining. The transect A is situated along a major passenger way and a creek in this area and therefore may be significantly affected by human activities (Pang et al. 2015). The transects B and C are relatively natural and should be excluded from significant human pollution. The sample locations include both vegetated and unvegetated areas. Therefore, these transects are suitable to investigate the accumulation of heavy metals by the plants and the resilience of plants to the heavy metal contaminations. In each site, duplicate *S. alterniflora* samples were collected and the adjacent sediment samples were also collected for referencing. Furthermore, offshore sediments (A5–A8, B8–B10, C7–C11) where plants were absent were also collected by the same method for comparison. The collected samples were stored in polyethylene bags and preserved in an icebox ($T = 10$–15 °C). The samples were taken back to laboratory for subsequent analyses.

Pretreatment of the samples

Digestion of the sediment samples

The sediment samples were dried in an oven at 50 ± 5 °C overnight to a constant weight and were ground in an agate grinder until homogeneous particles were obtained. Then, the samples were digested by concentrated HNO_3 and H_2O_2 (Fernandez-Cadena et al. 2014) using the following procedures: 1.00 g sediment sample was weighted and placed into flasks with distillation device. 10.0 mL (1:1) HNO_3 was added to each flask. Each mixture was gently shaken using a magnetic stirrer and then placed in an aluminum-heating block. The digestion of the sediment samples was performed as the following sequence of operations: heat the sample to 200 °C, stop heating to non-boiling, and reflux the mixture for 10–15 min. After cooling, 5 mL concentrated HNO_3 was added to the residual mixture and the mixture was heated at 200 °C for 30 min again. The above steps were repeated until there was no brown fume. Then, the solution was evaporated to about 5 mL in the state of non-boiling. After finishing the above steps, 2 mL distilled water and 3 mL H_2O_2 were added into the cooled sample, and the mixture was heated again till there was no bubble. The mixture was shaken gently and poured into polyethylene tubes. Then, it was diluted to 40 mL and centrifuged prior to elemental analyses.

Pretreatment of the plant samples

All of the plant samples were washed thoroughly by tap water to remove the attached impurities such as mud and sand. Then, each sample was divided into leaves, stems, and roots for our subsequent study. Each part was cut into small pieces by plastic scissors in order to avoid metal interference. Then, these pieces were washed thoroughly by distilled water (Phillips et al. 2015; Bonanno and Lo Giudice 2010). The samples were dried in a temperature-controlled oven at 50 ± 5 °C for 48 h and then were calcined in a muffle furnace (Nabertherm, L3/11/B180) at 500 °C for 90 min. The ashed plant samples were ground by a mortar for the following digestion procedures (Cacador et al. 2000; Padinha et al. 2000).

A portion of ashed plant samples (0.20 g) was weighted and placed into flasks with distillation device. Then, 10 mL mixture of HNO_3–$HClO_4$ (4:1 v/v) and a magnet were added into each flask. The mixture was gently shaken using a magnet and then placed in an aluminum-heating block. The operation procedures were as follows: heat the sample to approximately 100 °C, stop heating to non-boiling until nitrous fumes formed, then boil the mixture to approximately 200 °C. Repeat the above steps till there was no brown fume, and then evaporate the solution to about 5 mL in the state of non-boiling. After finishing the above steps, cool the sample, transfer it into a centrifugal tube, and dilute it to 50 mL with deionized water (Quan et al. 2007).

Elemental determination

The digested solutions from the sediment and plant samples were centrifuged at 3000 rpm for 5 min prior to the analyses of elemental concentrations. Metal concentrations (Co, Ni, Cd, Hg, Pb, Cu, and Zn) were determined by Inductively Coupled Plasma–Mass Spectrometry (ICP–MS, Thermo Fisher Scientific, XSeries II). The standard stock solution was diluted by 1% HNO_3 step by step. Some 10 $\mu g\ L^{-1}$ mixed standard solutions were measured to determine the sensitivity of the analyses. Reagent blanks, duplicate samples, and multi-elements standard solutions (Chinese national standard, GBS-04 series) were used for quality controls. The errors of the analyses as relative standard deviation were less than 10%. The detection limit (unit $\mu g\ L^{-1}$) of ICP-MS for each metal was: Co 0.0035, Ni 0.036, Cd 0.0051, Hg 0.00024, Pb 0.016, Cu 0.075, Zn 0.00024. The calculated detection limits (unit: $\mu g\ kg^{-1}$) for each metal were: in the sediment samples, Co 0.14, Ni 1.44, Cd 0.204, Hg 0.0096, Pb 0.64, Cu 3, Zn 0.0096; and in the plant samples, Co 0.875, Ni 9, Cd 1.275, Hg 0.06, Pb 4, Cu 18.75, Zn 0.06. The recoveries for all metals in both sediment and plant samples ranged from 90 to 110%. The operating parameters of ICP-MS were: 1200 WRF power and scanning mode of peak jumping, sampling time of 20 s with a dwell time of 1000 μs, sampling depth of 120 mm, and sample extraction yield of 1.0 mL min^{-1}. The total injection time for each sample was 60 s, and the cleaning time was maintained at 60 s. The flowing rates of cooling air, auxiliary gas, and atomization gas were 14.0, 0.75, and 0.92 L min^{-1}, respectively.

The physicochemical characteristics of the sediments

The temperatures (*T*, °C) of the sediments were measured in situ using a TES-1310 thermometer with a thermocouple (TES, Taiwan). The total organic carbon (TOC, %) and total nitrogen (TN, %) of the sediment samples were measured by a 2400 Series II CHNS/O Analyzer (PerkinElmer, US). The grain size of the sediments was measured by a Microtrac S3500 Laser Particle Size Analyzer (Microtrac, US). The measuring range and accuracy of the instrument were 0.02–2800 μm and 0.6%, respectively.

The pore water was extracted by a RHIZON 1921SA soil solution sampler with a diameter of 2.5 mm. The pH values of the sediments and pore water were measured by a TZS-pH-I pH meter (Tuopu, China). The pH calibration was performed using buffer solutions of pH 4, pH 7, and pH 10. The redox potential (Eh value) was measured by a ORP-401 m (Shanghai, China) with a platinum electrode.

Statistical analysis

Statistical analyses on the data were performed by a SPSS Windows release 18.0. Pearson's correlation coefficients were used to verify the relationships among variables.

Principal component analysis (PCA) was used to distinguish the associations among these elements (Armid et al. 2014). Bioaccumulation factors (BAF) were calculated to assess the accumulation of heavy metals by the *S. alterniflora*. The BAF was obtained by dividing the trace element concentrations in the plant samples by that in the sediments. The calculation equation is listed as follows:

$$\mathrm{BAF} = (X)_{\text{plant organ}}/(X)_{\text{sediment}} \tag{1}$$

where X refers to the concentration of the assessed heavy metal. BAF value of >1 indicates the significant accumulation of heavy metals by *S. alterniflora* from the sediments (Idaszkin et al. 2014).

Results and discussion

Heavy metal concentrations in the plant samples

Heavy metal concentrations in the different organs of *S. alterniflora* from the transects A, B, and C are given in Figs. 2, 3, and 4, respectively. The raw data can be found in Table S1. All of the samples exhibited higher concentrations of Cu and Zn with respect to other heavy metals. Both Cu and Zn were enriched in the stems and leaves. The accumulations of Cu and Zn in the aerial parts may be attributed to their roles in the plants. Cu and Zn play vital roles in the nutrition and enzymatic activities of plants (Bonanno and Lo Giudice 2010). Cu can be present in many oxidizing enzymes involving the redox processes of plants. In addition, Cu can also act as the components of chloroplast and participate in the electron transfer process during the photosynthesis (Lee et al. 2012). Zn is the metal activator of enzymes. It distributes in the chloroplast and promotes the formation of carbohydrates (Grill et al. 1989). In addition, Larsen (1983) and Lehtonen (1989) proposed that Zn is essentially important for the biosynthesis of the plant growth hormone indolyl-3-acetic acid, which is primarily active in the stems.

Co was mainly accumulated in the leaves with concentrations of less than 20 mg kg^{-1} in all three transects. Co is an essential nutrition element for plants and plays an important role for the plant physiology, such as the responses of stress and loads of process controls (Bush

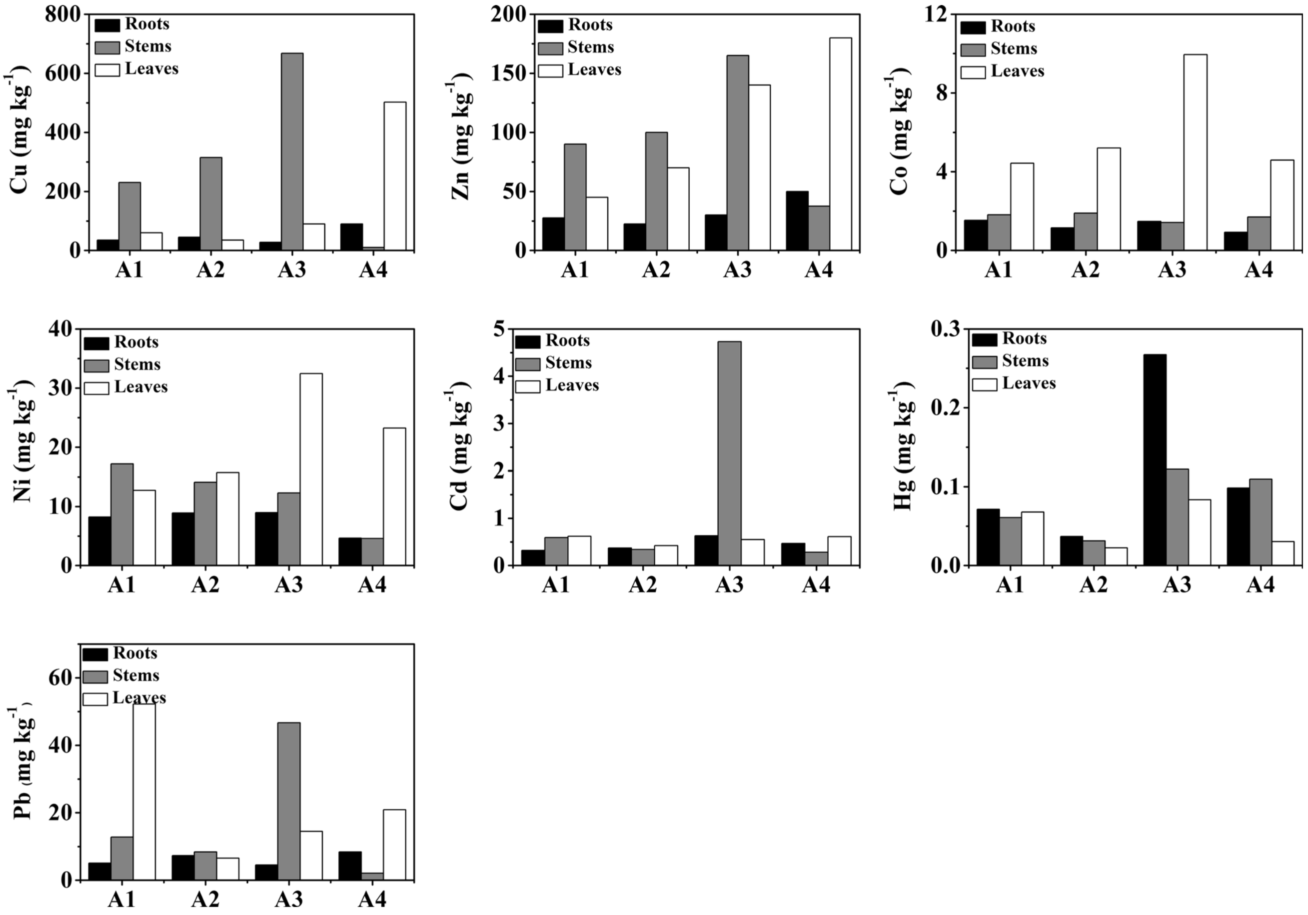

Fig. 2 Heavy metal concentrations in the roots, stems, and leaves of *S. alterniflora* in the transect A from the Andong tidal flat, Hangzhou Bay, China

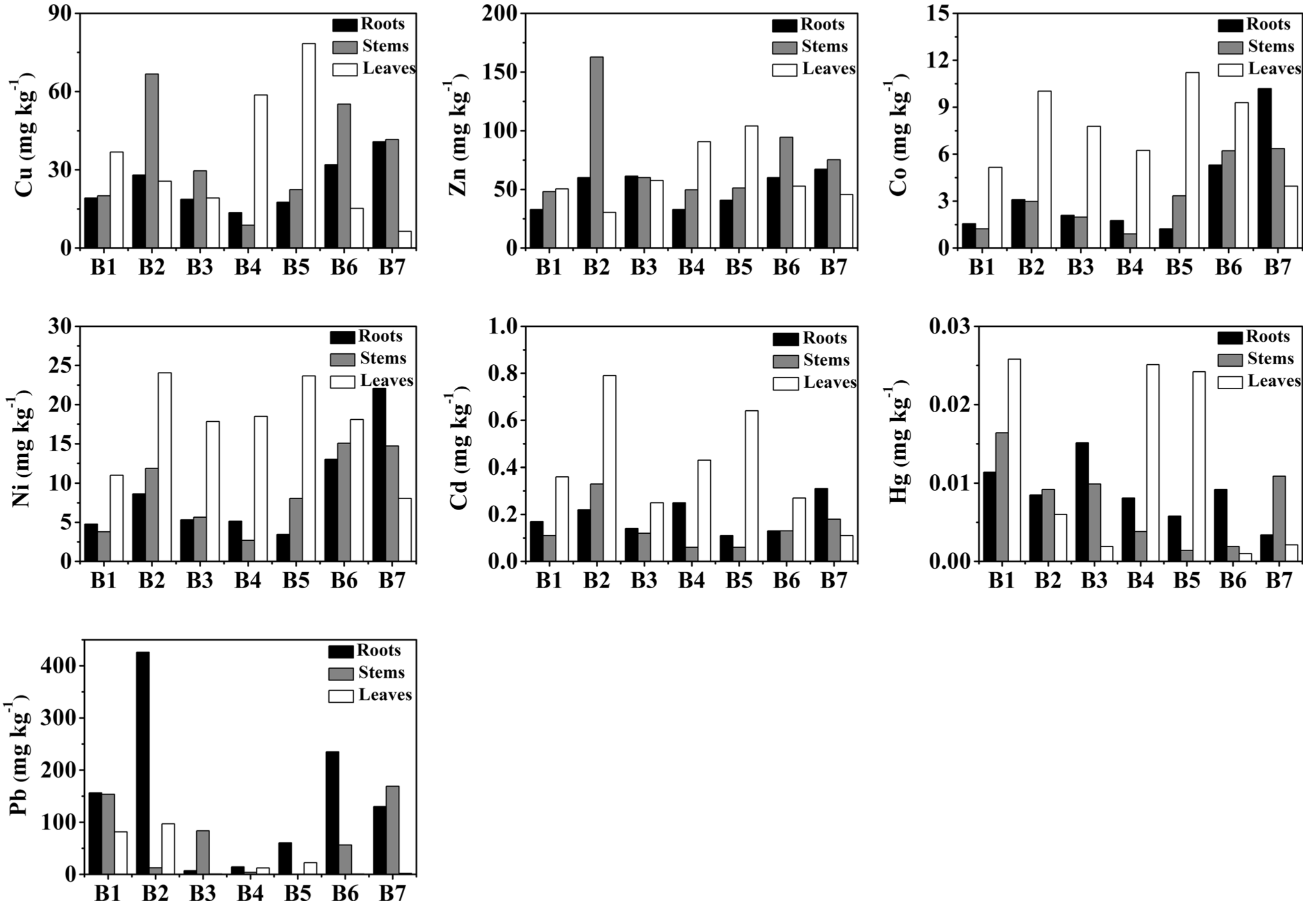

Fig. 3 Heavy metal concentrations in the roots, stems, and leaves of *S. alterniflora* in the transect B from the Andong tidal flat, Hangzhou Bay, China

1995). In addition, Co can fix the chloroplast membrane protein complexes, which are also concentrated in the leaves (Hajar et al. 2014). Ni was also mainly concentrated in the leaves of *S. alterniflora* with concentrations of 2–32 mg kg^{-1}. Ni is a toxic element for plants, and it can be significantly enriched in the leaves at the end of the growing period (Sainger et al. 2011). Therefore, it is reasonable to suggest that the accumulation of Ni in the plant leaves in this study may be ascribed to fact that we sampled mature plants in August and September.

Cd concentrations ranged from 0.03 to 4.73 mg kg^{-1} in the different organs of *S. alterniflora*. The distribution of Cd was relatively homogenous in all parts of the plants from the transect A and was mainly accumulated in the leaves in the transects B and C. Cd is a highly toxic and nonessential element, and it can hinder the growth and metabolism process of plants (Scholze et al. 1988; Divan Junior et al. 2009). Cd is rather mobile in the sediments, and it is readily available for plants (Madejon et al. 2004). It is suggested that Cd tends to be enriched in the aerial parts rather than the belowground parts of *S. alterniflora* (Hempel et al. 2008). Cd could also go into the root cells by competitive relationship with nutrients and then be transferred into the stems and leaves by leaf vacuoles (Almeida et al. 2004; Reboreda and Cacador 2007b; Vymazal et al. 2007). In addition, some studies suggested that ultrastructural modifications of aerial organs of plants in case of high Cd concentrations could lead to Cd accumulation in the stems and leaves (Pietrini et al. 2003).

Hg concentrations varied from below detection limit (<0.002 mg kg^{-1}) to 0.27 mg kg^{-1}. Hg concentrations were relatively homogenous in all parts of *S. alterniflora* with slightly enrichment in the roots of the samples from the transect A, and in the roots and leaves from the transects B and C. Hg is a toxic metal especially when its availability increased (Scholze et al. 1988). Recent reports suggested that the plants in aquatic environments preferably accumulate Hg in the roots (Fay and Gustin 2007; Zhang and Wang 2013), while early literatures reported that Hg was enriched in the leaves because the absorbed Hg can be quickly transported upwards (Shaw 1986; Lindberg et al. 1979). In addition, Hg enrichment in the leaves could also due to the Hg absorption from atmosphere by stomas (Ericksen et al. 2003).

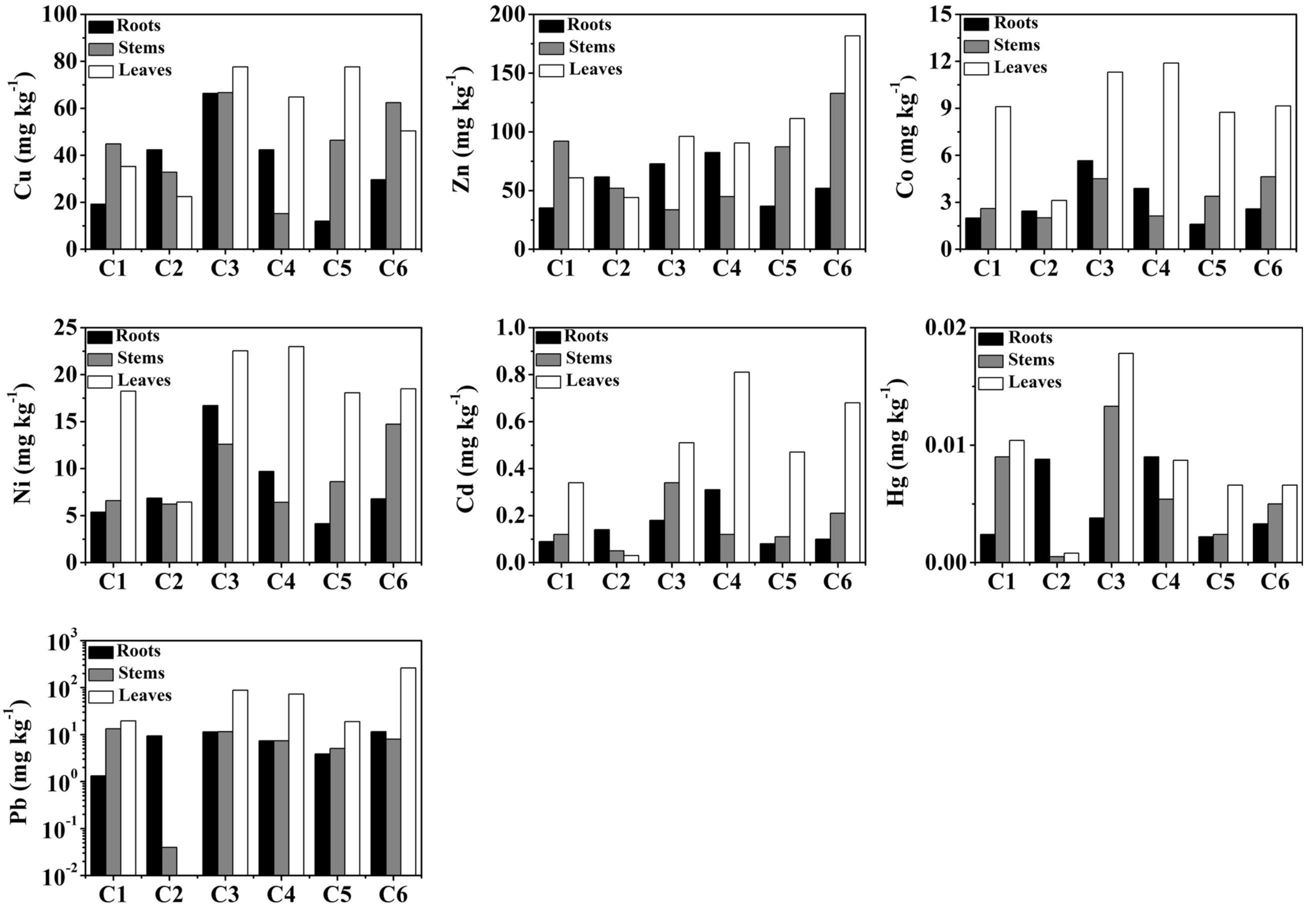

Fig. 4 Heavy metal concentrations in the roots, stems, and leaves of *S. alterniflora* in the transect C from the Andong tidal flat, Hangzhou Bay, China

Pb is considered as more toxic than other metals (Kabata-Pendias and Mukherjee 2007). Pb concentrations varied in three orders of magnitude among different samples. In the transects A and C, Pb was concentrated in the aerial parts, especially in the leaves. In the transect B; however, Pb was mainly enriched in the roots. Previous studies on the trace element accumulation in *Phragmites australis* showed that anthropogenic emission was a primary origin for Pb (Bonanno and Lo Giudice 2010). In addition, Pb accumulation in the leaves may be additionally affected by the exposure to the waste gas exhausted from automobiles (Schierup and Larsen 1981; Djingova et al. 2003; Bonanno and Lo Giudice 2010). Adjacent to our studied area, there are some factories (Fig. 1) producing mechanical and electrical equipment, auto parts, and nonferrous metals. These factories may act as sources for the heavy metals. In addition, Pb is an immobile element for its strong binding to organic matters and other components in plants (Aksoy et al. 2005; Mazej and Germ 2009).

The sediments from the transect A exhibited the highest heavy metal concentrations in the plants, while the transects B and C showed lower values, suggesting that the plants from the transect A were severely polluted by the assessed heavy metals (Pang et al. 2015). As shown in Fig. 5, Cu concentration in the transect A was elevated in all organs of the plant samples, Zn and Ni showed slightly increased concentrations in the stems and leaves, and Cd was especially accumulated in the stems. It is suggested that heavy metals could be adsorbed and accumulated by the plants, and some heavy metals may be eventually transported to the aerial parts of the plants according to their functions during metabolic processes. Hg concentrations in the transect A exhibited the highest values in the roots. It may be attributed to the severe contamination of Hg in the sediments, which overwhelmed the transportation ability of *S. alterniflora* (D'Souza et al. 2013; He et al. 2014; Wu et al. 2014). The Co concentrations in the plant samples from the transects B and C were slightly higher than those of transect A, while Co concentrations in the transect A sediments were much higher than those in the transects B and C (Table S2). This contradiction may be attributed to the fact that Co in the plants is limited by the metabolism of plants. Therefore, Co concentrations

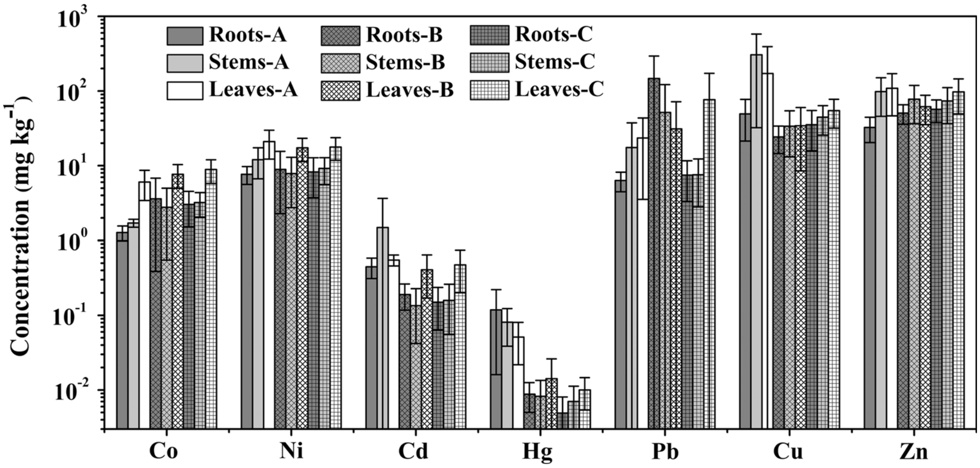

Fig. 5 Comparisons of the heavy metal concentrations in the roots, stems, and leaves of *S. alterniflora* from different transects in the Andong tidal flat, Hangzhou Bay, China. The root-A indicates the root samples from transect A

changed slightly with the background values (Bush 1995; Hajar et al. 2014).

Accumulation of heavy metals in the plants

In order to investigate the accumulation of heavy metals in the plants, we calculated the bioaccumulation factors (BAFs) of heavy metals by *S. alterniflora* (Idaszkin et al. 2014). As shown in Table 1, Co and Ni exhibited BAF values of less than 1 in most of the transects and organs, suggesting that the accumulations of Co and Ni by the plants were quite limited. Cd, Hg, Cu, and Zn showed BAF values between 1 and 5 in the transects B and C, indicating that these elements were significantly accumulated and were easily adsorbed by the plants (Bonanno and Lo Giudice 2010; Divan Junior et al. 2009; Zhang and Wang 2013). Most of the heavy metals presented BAF values of leaves > stems > roots, because these elements were essentially transported upward according to their functions during the metabolism of the plants. However, another possibility is that the *S. alterniflora* will be submerged by water during high tides. The leaves may additionally absorb metals from the water. As a consequence, the leaves presented enhanced BAF values with respect to the roots. An exception was Pb which exhibited BAF values of roots > stems > leaves, maybe because the Pb contamination in the transect B sediments has exceeded the transportation capability of the plants (Lisamarie et al. 2001; Windham et al. 2003). Note that all of the BAF values in the transect A were below 1 and were much lower than those in the transects B and C. It is ascribed to the fact that the transect A was a seriously polluted area, and the heavy metal contamination overwhelmed the accumulation capabilities of the plants (D'Souza et al. 2013; Wu et al. 2014). As a result, the BAF values were much lower although the heavy metal concentrations in the plants were significantly higher than those in the transects B and C.

The heavy metal distribution and accumulation in the plants may also be affected by the physicochemical characteristics of the sediments such as T, TOC, TN, pH, and grain size. As shown in Table S3, all of the sediment samples (and the pore water therein) from all transects exhibit relatively comparable physicochemical properties, although the samples from the transect A showed slightly higher T, lower TOC and TN, lower grain size, and lower pH values. Table S4 shows the correlations between the physicochemical properties and the heavy metal concentrations of the sediment samples. It is suggested that the temperature of the sediments was positively correlated (0.05 level) with Co, Cd, Hg, and Pb. The TOC, TN, pH,

Table 1 Bioaccumulation factors (BAF) of Co, Ni, Cd, Hg, Pb, Cu, and Zn in the *S. alterniflora* samples from the Andong tidal flat, Hangzhou, China

Site	Transect A			Transect B			Transect C		
BAF	Root	Stem	Leave	Root	Stem	Leave	Root	Stem	Leave
Co	0.01	0.01	0.04	0.48	0.44	**1.03**	0.30	0.32	0.88
Ni	0.02	0.03	0.05	0.57	0.57	**1.11**	0.38	0.42	0.73
Cd	0.04	0.13	0.05	**2.11**	**1.57**	**4.52**	**1.36**	**1.44**	**4.30**
Hg	0.05	0.03	0.21	**2.81**	**2.78**	**4.54**	0.95	**1.35**	**1.93**
Pb	0.02	0.04	0.06	**13.67**	**6.36**	**2.89**	0.08	0.08	0.86
Cu	0.10	0.61	0.34	**1.04**	**1.49**	**1.47**	**1.20**	**1.52**	**1.86**
Zn	0.04	0.13	0.14	**1.45**	**2.21**	**1.77**	**1.30**	**1.69**	**2.23**

BAF values of >1 are shown in bold

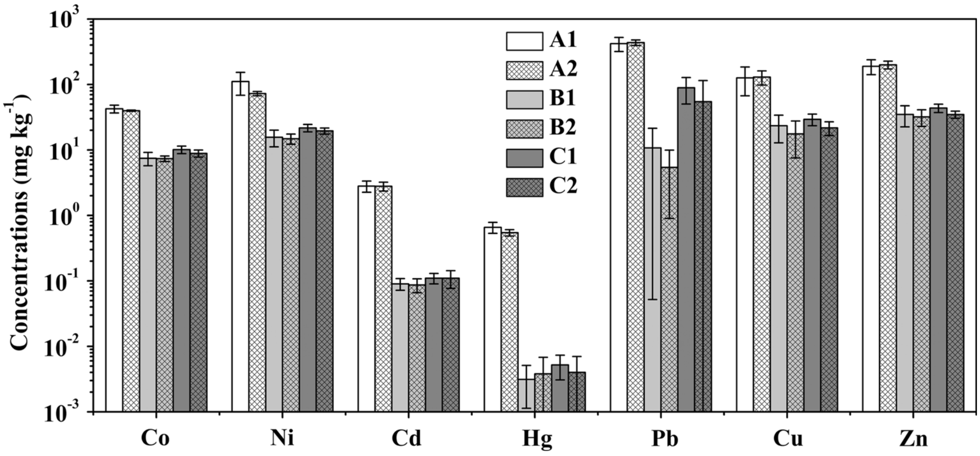

Fig. 6 Comparisons of the heavy metal concentrations of the sediments from the Andong tidal flat, Hangzhou Bay, China. A1—vegetated sediments from the transect A, B2—unvegetated sediments from the transect B

and grain size of the sediments were negatively correlated with their heavy metal concentrations, although the correlations were not significant. The pH and Eh of the pore water in the sediments, however, significantly (0.01 level) negatively correlated with almost all of the heavy metals. It is ascribed to the fact that the pH and Eh values control the balance between the sediment and the pore water, where lower pH values and a reducing environment facilitate the accumulation of heavy metals in the sediment.

These physicochemical properties can further affect the heavy metal accumulation in the plant samples. As shown in Table S5, the physicochemical properties of the sediments and the pore water therein did not show significant correlations with some of the heavy metals (Ni, Pb, and Zn) in the plants. Co positively correlated with TOC, TN, pH, and grain size, because Co is a nutrient for plants and it will be absorbed together with other nutrients. Cd, Hg, and Cu negatively correlated with the TOC and TN of the sediments and the pH and Eh values of the pore water. It is suggested that all of these heavy metals are biological toxic elements and they are unfavorable during the nutrition of the plants. In addition, the accumulations of these heavy metals were significantly affected by the pH and redox conditions of the pore water, which can change the speciation of these metals and consequently affect their bioavailabilities by the plants.

On the other hand, the bioaccumulation of heavy metals by the plants can in turn affect the heavy metal concentrations of the associated sediments. Although the plants can also absorb heavy metals from water besides the sediments, the heavy metal concentrations of the pore water within sediments are associated with the sediments. The heavy metals in the sediments and the pore water are in equilibrium and the metal concentrations of the sediments can represent the overall heavy metals that the plant can absorb (Doyle et al. 2003; Katsev et al. 2006; Santos-Echeandia et al. 2010) giving the fact that the physicochemical characteristics of the sediments are comparable. As shown in Fig. 6, however, the vegetation of the plants did not positively decrease the heavy metal concentrations of the associated sediments. The unvegetated sediments actually exhibited lower heavy metal concentrations than those with plants, although the plants do accumulate heavy metals from the sediments. This phenomenon was ascribed to two reasons: (1) The heavy metals removed by the plants were trivial for the total amounts of heavy metals in the sediments. Therefore, the heavy metal concentrations in the sediments were only slightly affected by the accumulation by plants. (2) The vegetated sediments are near shore and experienced stronger anthropogenic pollution and showed higher heavy metal concentrations with respect to the unvegetated sediments which located offshore.

Multi-statistical analyses

Pearson's correlation coefficients and PCA analyses are widely used to distinguish the correlations among elements and the sources of these elements. As shown in Table S6, almost all of the heavy metals in the sediments significantly positively correlated among each other. It is indicated that they were either derived from similar sources, or experienced analogous biogeochemical or accumulation processes (Ghandour et al. 2014; Maanan et al. 2015; Armid et al. 2014). In the *S. alterniflora* samples, however, only Co–Ni and Cu–Cd–Hg exhibited significant positive correlations (Table 2). The different correlations of heavy metals in the plants and sediments may be attributed to the differences in the bioavailability of trace metals for the plants. It is determined by many factors including the affinity of heavy metals, the speciation of heavy metals, the functions of heavy metals in the plants, the physicochemical properties of sediments or waters (Baldantoni et al. 2004; Kumar et al. 2006; Mishra et al. 2008; Zhang and Wang 2013). The Cd–Hg–Cu correlations and Co–Ni correlation in the plants suggested their analogous accumulation mechanisms in the plants, which is consistent

Table 2 Pearson's correlation coefficients of heavy metals in *S. alterniflora* from the Andong tidal flat, Hangzhou Bay, China

	Co	Ni	Cd	Hg	Pb	Cu	Zn
Co	1						
Ni	**0.670**	1					
Cd	−0.500	0.541	1				
Hg	−0.328	0.355	**0.904**	1			
Pb	0.446	0.261	−0.133	−0.201	1		
Cu	−0.302	0.410	**0.820**	**0.938**	−0.244	1	
Zn	0.249	0.531	0.507	0.423	−0.055	0.501	1

The statistically significant pairs ($P < 0.01$) are shown in bold

with previous arguments on the accumulation processes of these metals in the plants (Almeida et al. 2004; Reboreda and Cacador 2007b).

The PCA analyses showed similar results to the correlation analyses. As shown in Table 3, all of the heavy metals in the *S. alterniflora* can be grouped into two principal components, which occupied appropriately 79.30% of the total variance. The first component (PC1) generated 49.68% of total variance and exhibited high weights (>0.7) for Cd, Hg, and Cu, and moderate weights for Ni and Zn. The second component (PC2) accounted for 29.63% of the total variance, showing high weights for Co and Ni and moderate weight for Pb. Ni exhibited high loadings of >0.6 in both components. Trace metals in the sediments could be divided into two components (Table S7), accounting 86.87% of the total variance. The first component (PC1) produced 69.61% of the total variance and presented high weights (>0.7) for Co, Ni, Cd, Hg, Pb, and Zn, and moderate weight (0.62) for Cu. The second component (PC2) occupied about 17.28% of the total variance and exhibited high weight (0.76) for Cu (Bonanno and Lo Giudice 2010).

Table 3 PCA analyses of heavy metals in the *S. alterniflora* samples from the Andong tidal flat, Hangzhou Bay, China

	PC1	PC2
Eigenvalues	3.477	2.074
% of variance	49.676	29.626
Cumulative %	49.676	79.302
Eigenvectors		
Co	−0.052	**0.946**
Ni	**0.606**	**0.717**
Cd	**0.945**	−0.011
Hg	**0.922**	−0.272
Pb	−0.178	**0.658**
Cu	**0.925**	−0.242
Zn	**0.691**	0.314

PCA loadings >0.5 are shown in bold. The eigenvalues, percent of variance and cumulative, eigenvectors are given for the two principal components (PC1 and PC2)

Figure 7 shows the loading plots of heavy metals in *S. alterniflora* and in the associated sediments, respectively. The results showed that the heavy metals in the plants could be grouped into two parts using 0.6 as a boundary value: The first group includes Co, Pb, and Ni, and the second group is comprised of Cd, Hg, Zn, and Cu. The heavy metals in the sediment samples could also be divided into two groups, the single Cu group, and the second group comprised of Zn, Cd, Hg, Ni, Co, and Pb. These results suggested that (Co, Ni, Pb) and (Zn, Cu, Hg, Cd) behaved relatively coherently during the accumulation processes although they can be divided into different groups in the sediments. Furthermore, the two-factor cluster analysis (Fig. 7c) suggested that the plant and the sediment samples are not correlated, indicating that the bioaccumulation of the heavy metals was largely affected by the bioavailabilities of the heavy metals by the plants, which is consistent with former statements.

Conclusion

In summary, we collected marsh plant (*S. alterniflora*) samples and the associated sediments from the Andong tidal flat, Hangzhou Bay, and investigated the heavy metals accumulation in different organs of *S. alterniflora*. Most of the heavy metals including Co, Ni, Cd, Pb, Cu, and Zn were accumulated in the aerial parts (stems and leaves) of the plants. The heavy metal distributions were determined by their mobility in the plants and their roles during metabolic processes. With increasing heavy metal contaminations in the transect A, the accumulation of most of the heavy metals (such as Cu, Zn, Cd, Hg, and Pb) in the plants was significantly enhanced, while that of Co and Ni remained relatively constant. Most of the heavy metals except Pb exhibited BAF values of >1 and presented BAF values in the order of leaves > stems > roots in the transects B and C. In the transect A, however, most of the BAF values were less than 1, suggesting that the heavy metal contamination in the transect A may has exceeded the accumulation capabilities of the plants. PCA and correlation analyses indicated that Co–Ni and Cu–Cd–Hg behaved coherently during accumulation, while the correlations among other elements were disturbed due to their different accumulation mechanisms. The bioaccumulation of the heavy metals may be additionally affected by the physicochemical properties of the sediments and the pore water therein, which can influence the speciation and the behaviors of the heavy metals during nutrition and accumulation processes. Although the plants can adsorb and accumulate heavy metals from the sediments, the heavy metal contamination of the sediments cannot be relieved

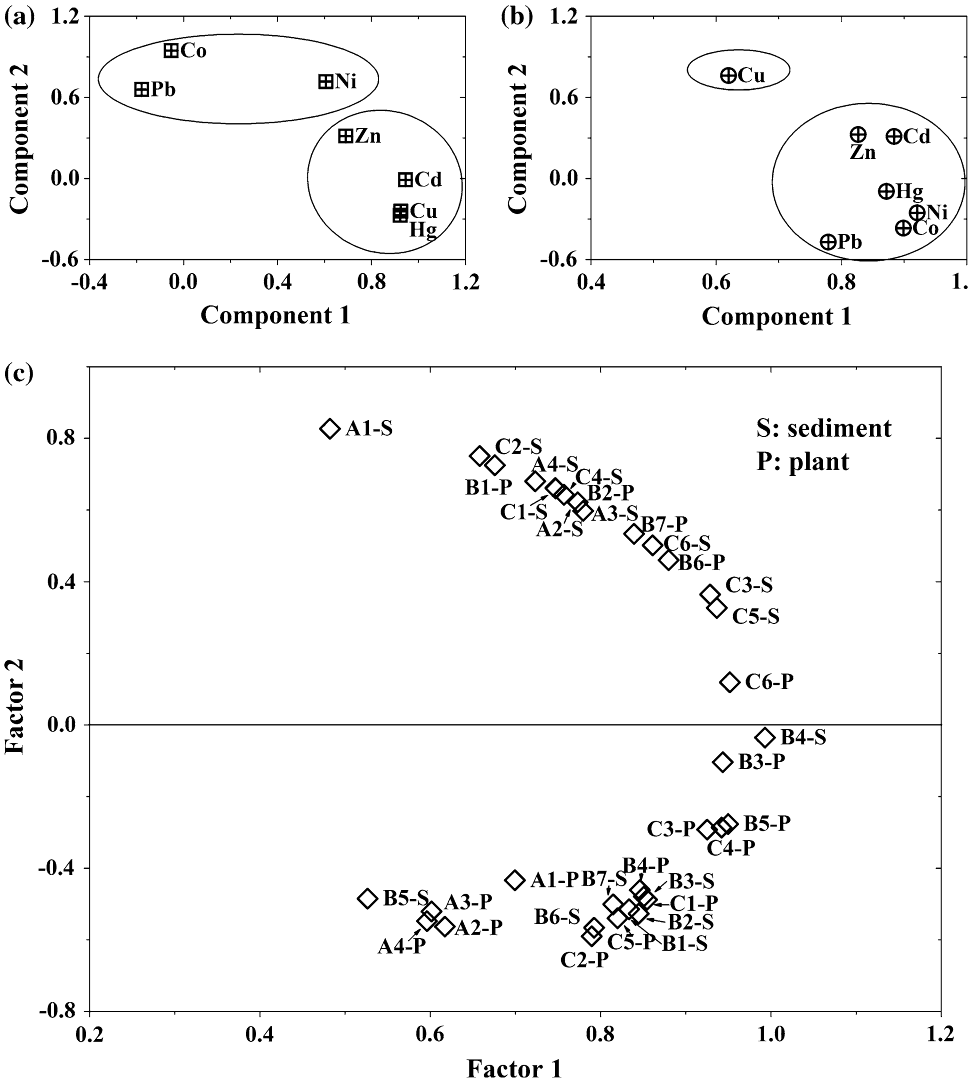

Fig. 7 Loading plots of the two components of heavy metals in **a** *S. alterniflora* and **b** the associated sediments from the Andong tidal flat, Hangzhou Bay, China. **c** Loading plots of the two factors of samples from the Andong tidal flat

since the sediments with dense vegetation showed analogous heavy metal concentrations to those of unvegetated sediments. This study provides essential information on the heavy metals accumulation by plants in estuaries, and we will investigate the accumulation mechanisms in depth in our subsequent study.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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