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Three tropical seagrasses as potential bio-indicators to trace metals in Xincun Bay, Hainan Island, South China*

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Abstract Concentrations of the trace metals Cu, Cd, Pb, and Zn were measured in seawater, rhizosphere sediments, interstitial water, and the tissues of three tropical species of seagrasses (*Thalassia hemprichii*, *Enhalus acoroides* and *Cymodocea rotundata*) from Xincun Bay of Hainan Island, South China. We analyzed different environmental compartments and the highest concentrations of Pb and Zn were found in the interstitial and seawater. The concentrations of Cd and Zn were significantly higher in blades compared with roots or rhizomes in *T. hemprichii* and *E. acoroides*, respectively. A metal pollution index (MPI) demonstrated that sediment, interstitial water, and seagrasses in the sites located nearest anthropogenic sources of pollution had the most abundant metal concentrations. There was obvious seasonal variation of these metals in the three seagrasses with higher concentrations of Cu, Pb and Zn in January and Cd in July. Furthermore, the relationships between metal concentrations in seagrasses and environmental compartments were positively correlated significantly. The bioconcentration factors (BCF) demonstrated that Cd from the tissues of the three seagrasses might be absorbed from the sediment by the roots. However, for *C. rotundata*, Zn is likely to be derived from the seawater through its blades. Therefore, the blades of *T. hemprichii*, *E. acoroides* and *C. rotundata* are potential bio-indicators to Cd content in sediment, and additionally Zn content (*C. rotundata* only) in seawater.

Keyword: metal contamination; seagrass; bioaccumulation; bio-indicator; South China Sea

1 INTRODUCTION

Reportedly, there are 60 species of seagrass that grow in shallow marine and estuary environments flanking all the continental margins except the polar regions (den Hartog, 1970; Hemminga and Duarte, 2000). These seagrasses are a primary food for many animals (e.g., manatee, dugong, green sea turtle) and form a critical habitat for thousands of other animal and plant species. In shallow-marine ecosystems seagrasses also play critical roles in the ecology and economy stabilizing sediment and carbon and nutrient cycling (Duarte, 1999; Dawes et al., 2004). Furthermore, hundreds of planktonic, epibenthic, and infaunal species depend on seagrass beds for survival with approximately 70% to 90% of commercial fish inhabiting seagrass during their life (Virnstein, 1987). However, several seagrass habitats in the world are now vulnerable and in degradation. Many have been completely destroyed by development of human populations in these coastal areas (Waycott et al., 2009).

Tropical seagrasses are found concentrated in two large areas worldwide: (1) the Indo-West Pacific and (2) the Caribbean and Pacific Coast of Central America (Green and Short, 2003). In China, ten genera and over twenty species of seagrass have been observed where there are two major distribution areas. These are the northern areas of the Liaoning, Hebei, Shandong and Fujian Provinces where the temperate species are distributed and the southern

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areas of Guangdong and Hainan Provinces, Guangxi Zhuang Autonomous Region, Hong Kong etc., where the subtropical and tropical species occur (den Hartog and Yang, 1990). The northern (e.g., *Zostera*, *Phyllospadix*) and southern (*Thalassia*, *Cymodocea*, *Enhalus*, *Halophila*, *Halodule*, *Syringodium*, *and Thalassodendron*) species belong to the North Pacific and Indo-West Pacific, respectively. Worldwide, seagrasses such as *Zostera marina* and *Posidonia oceanica* have been recommended as bioindicators of environmental conditions especially those in Mediterranean areas (Pergent-Martini and Pergent, 2000; Lewis and Devereux, 2009) and other tropical species (e.g. *T. hemprichii*, *E. acoroides* and *C. rotundata*) also require close attention.

Unlike pesticides and acid rain, trace metals exist naturally in marine environments, some of which (e.g. Cu and Zn) are essential micronutrients to organisms. However, at higher concentrations (10^{-5}–10^{-4} mol/L) of these metals including those essential micronutrients can be toxic to seagrasses (Ralph and Burchett, 1998; Prange and Dennison, 2000; Macinnis-Ng and Ralph, 2002). To date, there are few studies on seagrass from China and of those previous studies, they are mainly concentrated on morphology, taxonomy and spatial distribution (den Hartog and Yang, 1990; Huang et al., 2006; Shi et al., 2010). To our knowledge there is no reliable information on the levels of trace metals in seagrasses and their habitats in China.

The aims of this study were to: (i) determine, for the first time, the concentrations of four trace metals (Cu, Cd, Pb and Zn) in three tropical seagrass species (*E. acoroides*, *T. hemprichii* and *C. rotundata*) from Xincun Bay, Hainan Island, South China Sea; (ii) examine the relationship between these metal concentrations and their environmental compartments (seawater, rhizosphere sediment, interstitial water); (iii) reveal any metal accumulation patterns and evaluate the relevance of the seagrasses as potential bio-indicators of trace metals.

2 MATERIAL AND METHOD

2.1 Sampling and sample preparation

This study was performed in Xincun Bay (18°24′–18°27′N, 109°58′–110°2′E), located in southeast Hainan Island, South China Sea (Fig.1). This bay covers 22.6 km^2 of water surface and is a nearly closed lagoon with only one narrow entrance in the southwest, where it exchanges water with the South China Sea. Human activities such as waste

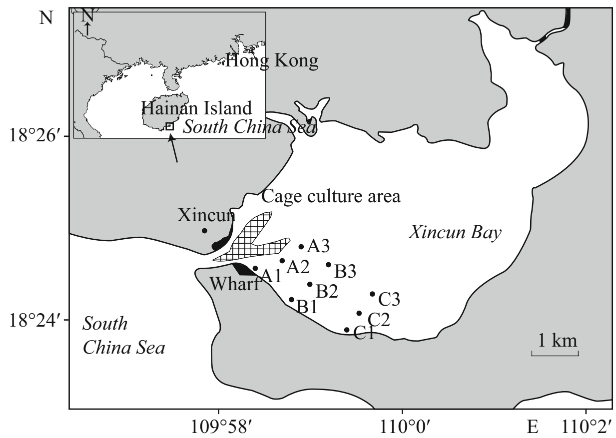

Fig.1 Sampling sites in Xincun Bay, Hainan Island, South China Sea

dischargement from Xincun City and transportation activities between the wharfs where a cage culture area is present are all potential sources of trace metals According to surveys performed in 2002, a mixed seagrass meadow with an area of about 200 ha inhabits the southern shallow waters of Xincun Bay. Five tropical seagrass species were observed at this meadow, *T. hemprichii*, *C. rotundata*, *Halodule uninervis*, *E. acoroides*, and *Halophilas ovalis* and *E. acoroides* and *T. hemprichii* were the dominant species (Huang et al., 2006).

In our study, we collected samples in July (Summer) 2009 and in January (Winter) 2010 to examine the seasonal dynamics of trace metals in seagrass. We chose only two sampling events because the bay is located in the tropical area of south China and seasonal change is not obvious. During these sampling periods in Xincun Bay, the measured bay water temperature ranged from 24–33°C, dissolved oxygen from 5.5 to 7.8 mg/L, salinity from 31 to 35, and pH from 7.8 to 8.3.

Our sampling sites are shown in Fig.1. Samples of rhizosphere sediments, seawater, and seagrasses (*E. acoroides*, *T. hemprichii* and *C. rotundata*) were collected from nine sites. These sites were distributed along the bay and at depths ranging from 0.1 to 1.0 m. Sites A_1, A_2, A_3 were near the mouth of the bay and close to the wharf and the cage culture area. Sites A_1, B_1, C_1 were in an area of high tide and all nine sampling sites were covered in a meadow of the three seagrass species (Fig.1). At each sampling site more than 10 individual seagrass plants were collected and subsequently grouped to form a composite plant sample. Three composite plant samples were prepared for each species at every sampling site. Seagrasses were harvested during low tide from 9 sites and following collection, their tissues were washed immediately in the bay water to wipe off remnant shells, sediments and debris. Epiphytes were carefully scraped from the leaves using a plastic spatula after which the samples were placed in sealed plastic bags and stored on ice. Only tissues from adult seagrass plants were used for analyses. For seawater and sediment samples, a mixed composite was used for analysis. Three replicates of seawater and sediment sample were analyzed. At each of the study sites the surface seawater was sampled in the meadow at the depth of ~0.1 m during high tide and stored in acid-washed bottles at 4°C before laboratory analysis. Rhizosphere sediments were collected with plastic core tubes (5 cm diameter and 30 cm long) during low tide and were pushed by hand into the sediment collecting ~10 cm of sample. The top 5 cm of the surface sediments were used for this research. Sediments were also stored in separate polyethylene bags, and then placed on ice until further processing. Interstitial water was centrifuged within 12 h from sediment samples following sediment collection. Polyethylene tubes were used during centrifugation for 15 min at 10 000 r/min at 4°C. The supernatant fluid was re-centrifuged for another 15 min to remove remaining suspended particles, and then the supernatant fluid was placed in polyethylene flasks in the dark at 4°C.

2.2 Laboratory methods

In the laboratory, the seagrass tissues were washed again using collected bay water and then with double distilled water to remove remaining debris, sediment etc. Each sample was then separated into blades (above ground tissues), rhizomes and roots (under ground tissues), placed on paper towels and aired at room temperature until partially dry. The tissues were then placed in an oven at 95°C for 24 h. After drying the seagrass tissues were crushed into powder using a ceramic pestle and mortar. The powdered tissues were stored in sealed plastic bags until analysis.

The residual seagrass leftover and shell material were removed manually from the rhizosphere sediments, and dried at 95°C for 24 h in an oven. The dry samples were gently grinded with a pestle and mortar, and the powdered sediments were then sieved to remove any residual tissues and shell material. The powdered sediment samples were also stored in sealed plastic bags before analysis.

All seawater samples were filtrated using Whatman glass fibre (0.45 μm) and acidified with 1.5 mL of concentrated HNO_3. All glass and plastic implements were cleaned with 3 mol/L HNO_3 and distilled water before use.

2.3 Trace metal analysis

The seagrass powder was weighed (500 mg) into an acid-washed (50 mL) conical flask for acid digestion. Concentrated nitric acid (5 mL) was added to the powder and heated at 70°C until a clear solution was formed. The temperature was then increased to 165°C and 1.0 mL of perchloric acid (35%) was added until a white fume evolved. The solution was cooled to room temperature, and the samples were filtered, diluted to 50 mL and then transferred into 50-mL centrifugal tubes for analysis.

This process was repeated for the powdered sediment samples of seagrass. The contents of Cu and Zn in all the samples were analyzed with a Varian Spectr AA 220 flame atomic absorption spectrometer (AAS). Cd and Pb were detected by AA 220 graphite furnace atomic absorption equipped with background correction. Seawater (GBW (E) 080040, China), sediment (GBW 07314, China) and plant (GBW 08517, China) reference materials were used to check the accuracy of chemical analytical procedure. Reagent blanks were also prepared to detect potential contamination during the digestion and chemical analyses. The instrument detection limits of seawater, sediments and seagrass were between 0.01 and 3.10 μg/L, 0.04 and 6.00, 0.005 and 2.00 μg/g, respectively. Dilutions of each element from 1 000 mg/L standard solutions were used as working standard solutions. The average metal recovery was 99% (reagent blanks), 99% (sample matrices) and 90% (reference materials).

2.4 Bioconcentration factors (BCF)

Bioconcentration factors used by Lewis et al. (2007) were based on trace metal concentrations in rhizosphere sediment samples and seagrass tissues and which we modified as follows:

$$\mathrm{BCF}=C_a/C_b$$

BCF can also represent trace metals in seawater/interstitial water (mg/L) and seagrass tissues (μg/g dry wt);

$$\mathrm{BCF}=C_a/C_m$$

C_a: metal concentration in seagrass tissues (μg/g dry wt)
C_b: metal concentration in rhizosphere sediments (μg/g dry wt)
C_m: metal concentration in seawater or interstitial water (mg/L)

2.5 The metal pollution index (MPI)

MPI modified by Usero et al. (2005) can be used to compare total metal content of seagrass between different sites. It was modified using the following equation:

$$\mathrm{MPI}=(C_1 \times C_2 \ldots C_n)^{1/n}$$

where C_n is the concentration of the metal n in seagrass or environmental compartments (seawater, sediment, and interstitial water) and n is the total number of metals. The MPI values indicate the metals pollution index from each site.

2.6 Statistical analysis

Statistical analyses of experimental data were performed using Origin 7.5 statistical software. Concentrations were compared between tissues, rhizosphere sediments, sites, seawater, seasonal variations and seagrass species by ANOVA. Where F values had a significant difference at $P<0.05$, the means from all samples were compared by using Tukey's studentized range test. The relationships between metal concentrations in tissues and environmental compartments were performed using Pearson's correlations analysis.

3 RESULT

3.1 Metals in seawater, rhizosphere sediment and interstitial water

The mean concentrations of Cu, Cd, Pb, and Zn in seawater ranged from 2.43 to 3.89, 0.06 to 0.56, 1.47 to 11.04 and 13.66 to 39.69 μg/L, respectively (Table 1). The Pb concentrations from all nine sampling sites and Zn concentrations in five sites out of nine exceeded the first class of seawater quality regulated by China's national standard GB3097-1997 (1.0 and 20 μg/L, respectively) (Table 1).

The concentration of trace metals from rhizosphere sediment of the seagrass bed show that all metals analysed except Cd (in A_1 and B_1) had concentrations less than the first class of sediment quality benchmark by the national standard GB18668-2002 (Table 2). Cd in the sediment ranged 0.20 to 0.62 μg/g dry wt

Table 1 Trace metal concentrations (μg/L) and MPI in seawater over the seagrass beds (mean±SE) [a]

Site	Trace metal				MPI
	Cu	Cd	Pb	Zn	
A_1	2.43±0.64	0.16±0.08	**2.78±2.14**	13.66±4.64	1.98
A_2	3.88±1.98	0.17±0.09	**8.04±7.56**	**31.69±7.58**	*3.60*
A_3	3.30±0.26	0.16±0.04	**3.02±1.35**	19.94±0.89	2.37
B_1	2.99±1.12	0.12±0.02	**3.02±0.38**	**22.70±10.92**	2.25
B_2	3.01±0.91	0.06±0.03	**1.47±0.70**	16.47±5.42	1.45
B_3	3.26±0.45	0.10±0.07	**2.92±0.18**	**26.78±0.58**	2.25
C_1	3.89±2.01	0.14±0.02	**2.23±1.49**	**28.18±9.41**	2.42
C_2	2.85±0.14	0.12±0.02	**1.82±1.06**	18.22±0.06	1.85
C_3	3.42±0.81	0.56±0.10	**2.28±1.56**	**29.58±3.40**	3.37
> MDL[b]	1.10	0.01	0.03	3.10	
GB 3097-1997 (I)[c]	≤5.0	≤1.0	≤1.0	≤20	

[a] $n=6$; [b] Method Detection Limit (μg/L); [c] First level of seawater quality criteria of China (μg/L). Values in bold exceed the first level and the maximum MPI values are in italic.

Table 2 Trace metal concentrations (μg/g dry wt) and MPI in sediments from seagrass beds (mean±SE) [a]

Site	Trace metal				MPI
	Cu	Cd	Pb	Zn	
A_1	7.98±3.92	**0.62±0.52**	13.71±5.08	63.87±22.46	*8.11*
A_2	2.56±0.01	0.36±0.34	8.69±1.34	12.72±0.40	3.19
A_3	2.34±0.01	0.42±0.40	6.71±1.32	24.40±13.02	3.56
B_1	2.52±0.21	**0.60±0.52**	6.89±0.20	24.37±12.48	3.98
B_2	1.01±0.76	0.34±0.32	5.85±0.25	6.61±0.54	1.90
B_3	1.86±1.10	0.46±0.44	6.51±0.95	56.56±48.14	4.20
C_1	3.60±1.26	0.46±0.44	9.68±0.72	26.88±0.77	4.55
C_2	3.23±1.13	0.20±0.18	4.13±2.63	13.60±3.36	2.45
C_3	1.44±0.16	0.32±0.30	7.07±0.39	6.99±2.99	2.18
> MDL[b]	0.50	0.04	3.00	6.00	
GB 18668-2002 (I)[c]	≤35.0	≤0.5	≤60	≤150	

[a] $n=6$; [b] Method Detection Limit (μg/g dry wt); [c] First level of sediment quality criteria of China (μg/g dry wt). Values in bold exceed the first level and the maximum MPI values are in italic.

Table 3 Trace metal concentrations (μg/L) and MPI in interstitial water from seagrass beds (mean±SE) [a]

Site	Trace metal				MPI
	Cu	Cd	Pb	Zn	
A_1	2.85±1.02	0.33±0.05	**7.37±2.29**	**50.70±13.80**	*4.31*
A_2	2.18±0.15	0.29±0.09	4.60±1.29	**58.98±19.02**	3.62
A_3	2.07±0.04	0.22±0.06	3.98±0.27	47.81±10.76	3.03
B_1	2.55±0.41	0.19±0.02	**7.62±2.55**	**52.30±14.77**	3.70
B_2	2.17±0.37	0.15±0.10	3.41±1.77	40.31±16.70	2.59
B_3	3.08±1.02	0.23±0.02	**7.87±0.99**	38.30±3.09	3.82
C_1	2.61±0.63	0.24±0.15	3.36±2.10	38.03±23.91	2.99
C_2	2.37±0.53	0.20±0.09	3.22±1.69	27.01±15.41	2.53
C_3	3.01±0.86	0.31±0.17	4.89±0.13	34.98±0.30	3.55
> MDL[b]	1.10	0.01	0.03	3.10	
GB 3097-1997 (I)[c]	≤5.0	≤1.0	≤1.0	≤20	
GB 3097-1997 (II)[d]	≤10	≤5.0	≤5.0	≤50	

[a] n=6; [b] Method Detection Limit (μg/L); [c] First level of seawater quality criteria of China (μg/L); [d] Second level of seawater quality criteria of China (μg/L). Values in bold exceed the second level and the maximum MPI are in italic.

with two sites exceeding the standard (0.5 μg/g dry wt).

Mean values of trace metal concentrations from interstitial water of the seagrass bed are presented in Table 3. Low concentrations of Cu (<4.0 μg/L) and Cd (<0.4 μg/L) were observed at nine sites. The mean values of Pb and Zn ranged from 3.22 to 7.87 and 27.01 to 58.98 μg/L, respectively. These values exceed the first level of China's national standard GB 3097-1997 (1.0 and 20 μg/L, respectively) at all nine sites and exceed the second level (5.0 and 50 μg/L, respectively) at three sites.

The ranges of MPI at nine sites of seawater, sediment and interstitial water were 1.45 to 3.60, 1.74 to 8.11 and 2.53 to 4.31, respectively, and the maximum MPI was located at sites A_2, A_1 and A_1, respectively (Tables 1–3).

We examined the seasonal variation from the metals Cu, Cd, Pb and Zn in seawater, sediment and interstitial water (Fig.2). The concentrations of all metals studied from seawater during July were lower compared with January, but there were no significant differences between the two seasons (P>0.05). However, Cd and Zn concentrations in sediment, and Cu, Cd, Pb and Zn concentrations in interstitial water displayed a January decrease, and significant seasonal differences were found for Cd content in sediment (P<0.05) and for Cu and Zn concentrations in interstitial water (P<0.01) (Fig.2).

3.2 Metals in seagrass

3.2.1 Comparison of sites, tissues and three different species

Spatial differences of trace metals in three seagrasses are shown in Table 4 The maximum mean values of the four metals analyzed in these seagrasses were all observed at site A_1, with the exception of Cd in *T. hemprichii* at site C. The MPI of the four metals in three seagrasses ranged 6.07–15.83 at nine sites and the maximum values were all observed at site A_1.

The boxplots in Fig.3 show the results from the trace metals analyzed in the different tissues of the three seagrass species. Significant differences between tissues were found for Cd in *T. hemprichii* and for Zn in *E. acoroides*, and concentrations of both these metals were higher in above ground tissues than in underground tissues of both species (P<0.05). Most of the between species mean trace metal contents, based on combined results for blades

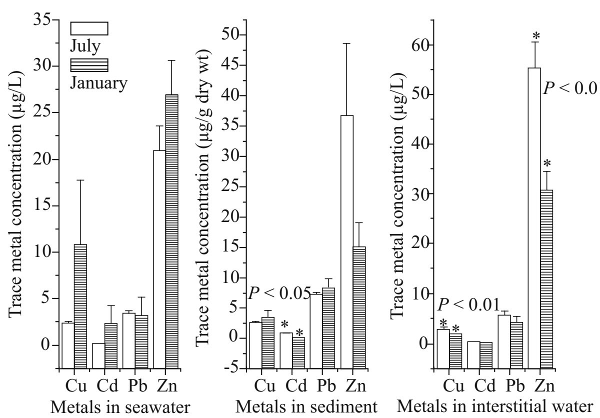

Fig.2 Seasonal variation of metals in seawater, sediment and interstitial water collected from seagrass beds in Xincun Bay

The bars represent the standard error (n=3). * significant difference of mean concentrations between seasons. P values refer to significant levels from the comparison performed by one-way analysis of variance.

Table 4 Trace metal concentrations (μg/g dry wt) and MPI of the three seagrasses (mean±SE) [a]

Site/ Species		Trace metal				MPI
		Cu	Cd	Pb	Zn	
A_1	1	**14.67±4.41**	2.19±0.70	**14.40±2.78**	**63.23±11.53**	*13.08*
	2	**20.27±4.47**	**3.94±0.84**	**18.50±2.65**	**35.69±10.06**	*15.15*
	3	**19.32±4.01**	**3.78±0.95**	**14.83±1.95**	**58.04±9.87**	*15.83*
A_2	1	11.07±4.26	3.17±0.32	9.22±1.70	39.33±13.34	10.62
	2	14.70±3.29	3.05±0.54	13.50±1.98	20.70±9.32	10.58
	3	13.40±3.29	3.46±0.87	12.63±1.54	35.63±10.32	12.02
A_3	1	7.25±1.76	2.11±0.59	11.82±1.98	49.72±9.55	9.74
	2	7.27±1.98	3.16±0.65	10.12±1.65	19.63±3.56	8.22
	3	6.10±1.69	3.58±0.58	10.89±1.39	21.65±4.21	8.47
B_1	1	10.80±4.08	2.39±0.77	13.89±3.30	54.68±9.25	11.83
	2	12.30±4.13	2.01±0.51	14.33±3.34	24.80±4.51	9.68
	3	15.15±3.28	1.97±0.39	12.98±3.14	45.45±8.87	11.52
B_2	1	4.04±1.78	3.32±0.08	6.77±0.90	15.38±2.18	6.11
	2	8.80±2.36	2.23±0.41	8.21±1.13	19.84±5.63	7.52
	3	7.70±1.36	2.35±0.64	7.67±1.08	20.15±3.21	7.27
B_3	1	8.82±2.95	2.06±0.66	11.11±2.51	51.87±16.91	10.12
	2	5.63±1.32	1.92±0.35	11.90±2.31	25.32±8.31	7.55
	3	13.65±3.01	2.06±0.57	10.35±2.07	37.32±7.98	10.21
C_1	1	8.76±2.86	2.05±0.66	13.09±3.40	45.95±14.46	10.19
	2	9.60±2.46	2.32±0.34	13.01±3.13	32.65±12.21	9.86
	3	14.63±6.32	2.18±0.48	12.38±2.68	46.80±10.32	11.66
C_2	1	3.76±1.29	**3.79±0.18**	6.89±0.17	13.85±6.45	6.07
	2	8.65±2.54	2.12±0.42	8.80±0.51	10.56±6.32	6.43
	3	9.92±2.35	2.31±0.43	7.50±0.86	12.98±8.98	6.87
C_3	1	8.75±2.66	1.75±0.56	12.48±3.46	39.55±13.78	9.32
	2	12.32±4.21	1.95±0.53	10.80±2.01	26.63±12.32	9.12
	3	16.26±3.21	1.77±0.64	13.20±2.10	30.56±11.32	10.38
> MDL[b]		2.00	0.005	0.04	0.40	

1. *T. hemprichii*; 2. *E. acoroides*; 3. *C. rotundata*; [a] $n=6$; [b] Method Detection Limit (μg/g dry wt.); The maximum values of each species of seagrass are in bold and the maximum MPI are in italic.

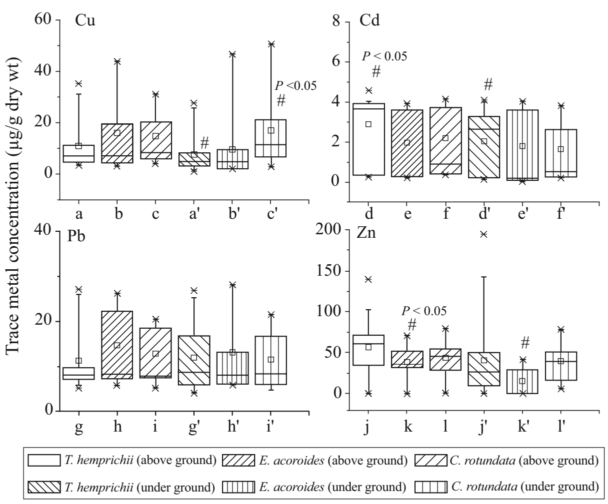

Fig.3 Boxplots of metal concentrations in different tissues and three different species of seagrass from Xincun Bay

'—' represents the max and min; '□' represents the mean ($n=6$), '×' represents the percentile of 99% and 1% and '#' represents significant differences of mean concentrations between tissues and species. P values show significant levels from the comparison performed by one-way analysis of variance.

and roots/rhizomes, were not significantly different. However, Cu content in roots/rhizomes of *C. rotundata* was significantly higher than that of *T. hemprichii* ($P<0.05$).

3.2.2 Comparison of seasons

The seasonal variation of metal concentrations between the three seagrasses are shown in Fig.4. The data from each season was calculated from all nine sampling sites. The histograms demonstrate clear seasonal variation of metal concentrations. The accumulation of Cu, Pb and Zn were significantly higher in January than in July in all three seagrasses ($P<0.01$ or $P<0.05$). In contrast significantly higher concentrations of Cd in the seagrasses were observed in July ($P<0.01$) (Fig.4).

3.3 Relationship of metal contents between tissues of seagrasses and environmental compartments

For *T. hemprichii*, significant positive correlations were observed between Cu and Cd concentrations in sediments and root/rhizomes ($P<0.05$; the correlation

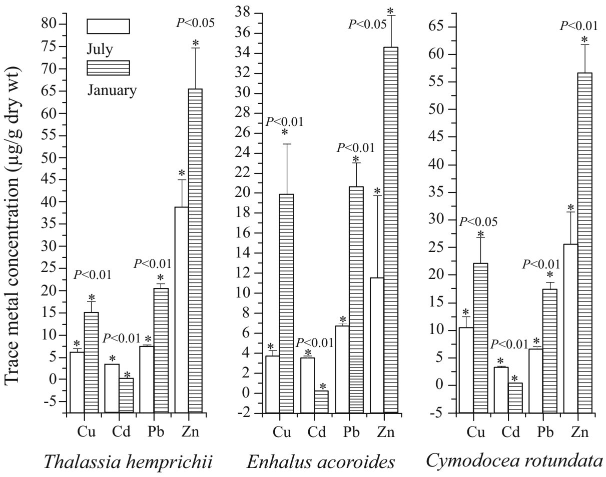

Fig.4 Seasonal variation of metal concentrations in three species of seagrass from Xincun Bay

The bars represent the standard error (n=3). * significant difference of mean concentrations of each metal between seasons. P values show significant levels from the comparison performed by one-way analysis of variance.

coefficient R=0.81 and 0.95, respectively), and with Cd and Zn between sediments and blades (P<0.05; R=0.94 for Cd), (Table 5).

For *E. acoroides*, Cu in roots/rhizomes were significantly positively correlated with those in seawater (P<0.05). Additionally a positive correlation for Cd was found between tissues (blades) and rhizosphere sediments (P<0.05; R=0.98 for Cd in blades). Zn concentrations in tissues were positively correlated with those in seawater and negatively correlated with those in rhizosphere sediments and interstitial water, however, neither of these were statistically significant (P>0.05) (Table 5).

For *C. rotundata*, only Zn concentration in blades were significantly positively correlated with those in seawater (P<0.05; R=0.85). For Cd, significant positive correlation was found between blades and rhizosphere sediment (P<0.05, R=0.94 for Cd in blades); and for Cu a significant negative correlation was observed between blades and interstitial water (P<0.05) (Table 5).

To clarify these findings, the significantly positive correlations were illustrated separately in Fig.5. No significant relationship was found for Pb between seagrass tissues and environmental compartments.

3.4 Bioconcentration factors (BCF)

BCF based on Cu, Cd, Pb, and Zn in rhizosphere sediments and three seagrass tissues had higher values of 9.76 in roots and rhizomes of *C. rotundata*;

Table 5 Correlation (correlation coefficient, *R*) between metal concentrations in tissues (blades and roots/rhizomes) of the three seagrass species and in seawater/rhizosphere sediment/interstitial water, and the mean values of bio-concentration factors (BCF), in parentheses

Trace metal (correlation coefficient (*R*) and bio-concentration factor (BCF) (in parenthesis))						
Specification			Cu	Cd	Pb	Zn
T. hemprichii	Seawater	Blades/seawater	0.29 (3556)	-0.27	-0.14	0.45 (3059)
		Roots and rhizomes/seawater	-0.12	-0.27	-0.04	-0.23
	Sediment	Blades/sediment	0.38 (3.90)	0.94*(8.65)	0.08 (2.52)	0.49*(6.04)
		Roots and rhizomes/sediment	0.81*(2.88)	0.95*(6.44)	0.34 (2.43)	0.36 (2.79)
	Interstitial water	Blades/interstitial water	-0.43	0.39 (8705)	-0.38	-0.37
		Roots and rhizomes/interstitial water	-0.18	0.34 (7145)	-0.13	-0.02
E. acoroides	**Seawater**	Blades/seawater	0.19 (3713)	-0.52	-0.20	0.67(1527)
		Roots and rhizomes/seawater	0.96*(1670)	-0.51	0.02 (9786)	0.79 (668)
	Sediment	Blades/sediment	0.75 (5.94)	0.98*(6.34)	0.01 (2.04)	-0.75
		Roots and rhizomes/sediment	0.31 (3.72)	0.96*(5.68)	0.28 (2.34)	-0.70
	Interstitial water	Blades/interstitial water	-0.74	0.49(7654)	-0.46	-0.39
		Roots and rhizomes/interstitial water	-0.69	0.53 (7721)	-0.51	-0.89*
C. rotundata	**Seawater**	Blades/seawater	0.28 (3538)	-0.55	0.11 (6688)	0.85*(1705)
		Roots and rhizomes/seawater	0.06 (6309)	-0.48	-0.20	0.79 (1700)
	Sediment	Blades/sediment	0.57(6.22)	0.94*(10.70)	0.39 (1.71)	-0.76
		Roots and rhizomes/sediment	0.67(9.76)	0.95*(9.24)	0.12 (1.75)	-0.81
	Interstitial water	Blades/ interstitial water	-0.82*	0.61 (8536)	-0.36	-0.60
		Roots and rhizomes/interstitial water	-0.78	0.54 (7795)	-0.73	-0.72

Significance level: *P<0.05.

BCF values were not calculated while metal concentrations in tissues were negative correlated with seawater/ rhizosphere sediment/interstitial water.

Words in bold mean the main source of metals accumulated in that genus of seagrass.

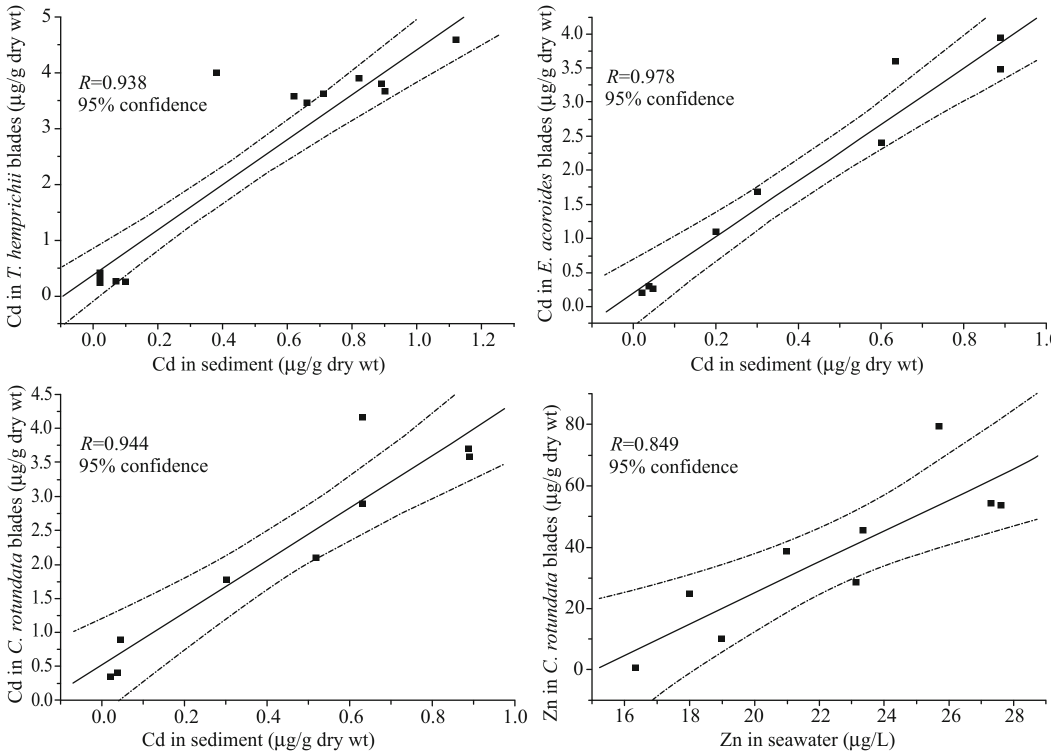

Fig.5 Concentrations of Cd and Zn in blades of *T. hemprichii*, *E. acoroides* and *C. rotundata* plotted against the concentrations measured in sediments and seawater

10.70 in blades of *C. rotundata*, 2.52 in blades of *T. hemprichii* and 6.04 in blades of *T. hemprichii*, respectively.

Based on Cu and Zn in seawater and the blades of three seagrasses, BCF values had higher values of 3 713 (*E. acoroides*) and 3 059 (*T. hemprichii*), respectively. The Cd in interstitial water and seagrass tissues reached the highest BCF value of 8 705 from the blades of *T. hemprichii* (Table 5).

4 DISCUSSION

Largely, trace metal contents in environment compartments (seawater, rhizosphere sediments and interstitial water) from Xincun Bay obtained in this work are intermediary to previous reports worldwide, see Table 6. For example, Cu concentrations from seawater in this study ranged between 2.43 and 3.89 μg/L, (with nine sites <5 μg/L). These were much higher than results obtained by Campanella et al. (2001), from an uncontaminated area in the Mediterranean Sea (0.51–0.82 μg/L). However, our results were much lower than those reported from a highly human-impacted area in the Antikyra Gulf, Greece (23–74 μg/L) (Malea and Haritonidis, 1995). Concentration of metals in rhizosphere sediments in this study were intermediary to previous reports (Ward et al., 1986; Malea and Haritonidis, 1995; Schlacher-Hoenlinger and Schlacher, 1998; Lafabrie et al., 2007). Very few metal concentrations in interstitial water of seagrass beds have been previously reported. In our study, Pb and Zn concentrations in interstitial water exceeded 1.0 to 5.0 and 20 to 50 μg/L, respectively, while Cu and Cd were not obviously elevated. Similar results were reported by Doyle et al. (2003) in the interstitial water of the contaminated Lake Macquarie, Australia.

In our study, the content of Pb in seawater was up to eight times higher than the national GB3097-1997 benchmark, and less than two times for Zn. In addition, Pb concentrations at all nine sites and Zn at five of nine sites exceeded the criteria. This suggested that Pb and Zn were the two most abundant metal pollutants, particularly Pb in the interstitial and seawaters water of Xincun Bay. The maximum MPI for seawater and interstitial water were observed at stations A_2 and A_1, respectively. These two sites are in the high tide belt and close to the wharf, the cage culture area and the town of Xincun, hence they are more likely to be affected by anthropogenic

Table 6 **Examples of reported trace metal concentrations in the environment of seagrass beds and in seagrass tissues[a]**

Environment/Tissue	Species	Trace metal				Location	Reference
		Cu	Cd	Pb	Zn		
Seawater (μg/L)	*Cymodocea nodosa*	23–74	0.7–4.0	3.0–24	24–93	Antikyra Gulf (Greece)	Malea and Haritonidis (1995)
	Posidonia oceanica	0.51–0.82	0.10–0.18	0.32–0.81	1.76–5.00	Favignana Island (Italy)	Campanella et al. (2001)
	Posidonia oceanica	-	<0.01–0.02	0.038–0.075	-	North-western Mediterranean (Italy)	Lafabrie et al. (2007)
	Thalassia testudinum, *Halodule wrightii*	13.9–61.1	<1.0	<0.6–16.7	<3.0–66.4	Florida seagrass beds (USA)	Lewis et al. (2007)
	Thalassia hemprichii, *Cymodocea rotundata*, *Enhalus acoroides*	1.8–8.2	0.03–0.9	0.2–15.6	9.0–47.3	Xincun Bay, Hainan Island (China)	This study
Sediment (μg/g dry wt)	*Posidonia australis*, *Posidonia sinuosa*	-	0.2–267	2.0–5270	11–16700	Port Pirie (Australia)	Ward et al. (1986)
	Cymodocea nodosa	5.2–397	0.3–87	22.5–633	12.3–69.8	Antikyra Gulf (Greece)	Malea and Haritonidis (1995)
	Posidonia oceanica	<25	<0.5	134	<80	Gulf of Naples (Italy)	Schlacher-Hoenlinger and Schlacher (1998)
	Thalassia testudinum	3.8–14.0	-	1.8–10.1	27.1–80.9	Lower Laguna Madre (USA)	Whelan et al. (2005)
	Posidonia oceanica	-	0.03–0.40	4.67–44.5	-	North-western Mediterranean (Italy)	Lafabrie et al. (2007)
	Thalassia testudinum, *Halodule wrightii*	<0.08–70.8	<0.2–0.39	0.21–28.5	2.2–50.4	Florida seagrass beds (USA)	Lewis et al. (2007)
	Thalassia hemprichii, *Cymodocea rotundata*, *Enhalus acoroides*	0.8–11.9	0.02–1.14	1.5–18.8	6.1–104.7	Xincun Bay, Hainan Island (China)	This study
Interstitial water (μg/L)	*Heliocidaris tuberculata*	<1–7	<1	<4–9	5–40	Lake Macquarie (Australia)	Doyle et al. (2003)
	Thalassia hemprichii, *Cymodocea rotundata*, *Enhalus acoroides*	1.8–4.1	0.05–0.48	1.66–10.17	11.6–78.0	Xincun Bay, Hainan Island (China)	This study
Leaves (μg/g dry wt)	seven species	2.2–9.8	0.13–1.20	0.84–6.00	2.2–9.8	the Flores Sea (Indonesia)	Nienhuis (1986)
Leaves	*Posidonia oceanica*	2.8–148	2.7–44.0	10.5–123	27.1–97.7	Antikyra Gulf (Greece)	Malea et al. (1994)
Leaves	*Posidonia oceanica*	5.0–21.8	1.1–3.1	0.6–10.0	102–165	Favignana Island (Italy)	Campanella et al. (2001)
Blades (μg/g dry wt)	*Posidonia oceanica*	-	-	1.6–3.1	2.4–3.0	north-western Mediterranean (France, Italy)	Lafabrie et al. (2009)
Blades	*Thalassia hemprichii*	9.0–12.8	2.6–3.2	9.8–12.6	49.7–63.6	Xincun Bay, Hainan Island (China)	This study
	Cymodocea rotundata	10.6–19.1	1.5–2.9	10.0–15.5	32.7–54.6		
	Enhalus acoroides	9.8–22.3	1.2–2.7	11.1–18.2	28.9–48.0		

a Ranges from various sites or different time periods.

activities. Waste from fishing vessels at the wharf and the cage culture area (e.g. waste batteries) and discharges from shipyards in the town are important potential point sources of Pb and Zn. In interstitial water Pb and Zn were also the two most abundant metal pollutants. However, Cd concentrations in sediment at two sites exceeded the first level of the national standard GB18668-2002, indicating that the sediment in the bay was slightly polluted by Cd.

According to the combined results of both blades and roots/rhizomes of three tropical species in our study, the concentrations of the four metals across species were not significantly different. Cu in the roots and rhizomes was an exception, being significantly higher in *C. rotundata* than *T. hemprichii*. This indicated that the roots of *C. rotundata* were more efficient at taking up Cu than those of the other two species. High BCF values based on concentrations of Cd in the rhizosphere sediments and tissues of three seagrass species, suggested that the translocation and accumulation of Cd from sediment to seagrass tissues occurs in all three of these tropical seagrasses.

We compared metal concentrations from our seagrasses with those from other geographical areas. For example, Pb concentrations in *T. hemprichii*, *E. acoroides* and *C. rotundata* (9.8 to 12.6, 11.1 to 18.2 and 10.0 to 15.5 μg/g dry wt, respectively from our study) were higher than those from of *P. oceanica* (0.6 to 10.0 μg/g dry wt) measured in the north-western Mediterranean Italy, (Campanella et al., 2001), yet lower than results of *P. oceanica* (10.5 to 123 μg/g dry wt) detected in Antikyra Gulf, Greece (Malea et al., 1994). Hence, Cu, Cd, Pb and Zn concentrations in seagrasses from Xincun Bay, South China Sea were higher than those from places that are regarded as uncontaminated (Nienhuis, 1986; Campanella et al., 2001), but lower than those from areas strongly affected by human activities (Malea et al., 1994; Malea and Haritonidis, 1995).

No significant seasonal variation of metal concentrations in the environment of Xincun Bay was found. However, significant seasonal variation of metals in seagrass tissues was observed. Cu, Pb and Zn concentrations were higher in January than in July. This may be explained by the growth dilution of the metal concentrations per unit mass of seagrasses. Also an obvious decline of Cu, Cd, Pb and Zn at the beginning of the growth season was detected in other seagrass species (Mudroch and Capobianco, 1979; Lyngby and Brix, 1982; Ward, 1987; Capiomont et al., 2000). However, our observations showed Cd concentrations in three seagrasses were significantly higher in July than January. This finding is consistent with previous literature (Malea et al., 1994; Malea and Haritonidis, 1995), and represents the age-dependent distribution pattern of this metal (Malea, 1994). In January, adult blades tend to senesce and are shed from the seagrass, while young blades appear to be more numerous. In July however, after rapid growth in spring, adult blades are in the majority (Malea and Haritonidis, 1995). Furthermore, Lyngby and Brix (1989) and Ancora et al. (2004) both reported a parallel increase of Cd concentration in tissues with the tissue age of the seagrass. These explanations seem to coincide with the result of this study regarding higher Cd concentrations in adult blades in summer. Meanwhile, high Cd concentrations in seagrasses and other macrophytes can induce the synthesis of metal-biomolecule proteins such as phytochelatin and metallothionein which can reduce the oxidative stress caused by metals (Cozza et al., 2006; Alvarez-Legorreta et al., 2008; Wang et al., 2010).

We found significant positive correlations between Cd in tissues of three seagrasses and in sediments as well as between Zn in blades of *C. rotundata* and in seawater. This suggests that the distribution of these metals in seagrass seems to be affected by their similar distribution of environmental compartments, especially for Cd and Zn which had significant positive correlations. Similar results were found between blades and sediment in other species of phanerogams (Mudroch and Capobianco, 1979; Pulich, 1980; Lyngby and Brix, 1987; Tiller et al., 1989; Thangaradjou et al., 2010). These results show that Cd can be acropetally transferred and accumulated in blades of seagrasses. This is consistent with results observed in *Elodea canadensis* and *Z. marina* (Mayes et al., 1977; Malea et al., 2008). In three seagrasses, Zn concentrations were higher in blades than roots and rhizomes. It seemed to be the situation with *E. acoroides*, where significant differences were found. This is consistent with the hypothesis that a possible uptake of Zn from seawater into the blades. Similar results were found by Warnau et al. (1996) and Peng et al. (2008). They reported that Cd and Zn could be linearly absorbed from ambient seawater by seagrass shoots. However, in our study, this situation could have only occurred with *C. rotundata*. If this occurred, it would be consistent with results reported by Malea et al. (1994) and Whelan et al. (2005). Hence, preferential accumulation of Cd and Zn in blades is possible. Cd may be absorbed by roots from rhizosphere sediment and then was acropetally translocated, while Zn might be absorbed from seawater by blades of *C. rotundata* and then transported basipetally. These results are consistent with those of Lafabrie et al. (2008) and Peng et al. (2008).

Lyngby and Brix (1987) and Lafabrie et al. (2007) had reported that seagrasses are sedentary, sensitive to environmental variations, behave as low stages of the food chain, and react more rapidly to the presence of pollutants than organisms at higher stages (e.g. *Mytilus galloprovincialis*). Moreover, sediments are considered as a sensitive indicator of human-induced impact (Calmano et al., 1996; Acevedo-Figueroa et al., 2005). In our study, the high values of BCF were observed based on Cd in blades of three seagrasses and sediment; and Zn in blades of *C. rotundata* and seawater. Meanwhile, a significant positive correlation was found for Cd between blades of three seagrasses and sediment; and for Zn between blades of *C. rotundata* and seawater. Based on the results presented above, we suggest that *T. hemprichii*, *E. acoroides* and *C. rotundata* are potentially valuable bio-indicators to Cd contamination in sediment whereas *C. rotundata* may be a potential indicator to Zn in seawater of tropical seagrass bed.

5 CONCLUSION

Low metal contamination was found in Xincun Bay, in the South China Sea, namely from Pb and Zn in seawater and interstitial water and Cd in sediment. Metals concentrations from our study were intermediary to previously reported sites from other locations. Sites A_1 and A_2 in our study were the most contaminated. These sites are heavily affected by human activities and were the closest to the wharf, shipyard and the cage culture area in Xincun. Similarly, the maximum values of metals accumulated in the tissues of the three tropical seagrasses studied were also determined at site A_1. There was a clear seasonal variation of metals in seagrasses where the accumulation of Cu, Pb and Zn was significantly higher in January than July, whilst Cd was significantly higher in July than January. The variability of these metal concentrations in seagrass tissues could be due to different metal uptake of different seagrass species. It could also be due to different environmental factors (e.g. temperature variation, pH, and salinity) which requires further detailed studies.

We also suggest a preferential accumulation of Cd and Zn in blades rather than in roots and rhizomes is possible. The Cd concentrations in the three tropical seagrasses from this study may be absorbed by roots and then transported acropetally while Zn may be absorbed by blades from seawater and then transported basipetally. Hence, we propose the use of blades of (i) *T. hemprichii*, *E. acoroides* and *C. rotundata* as bio-indicators to Cd in sediment; and (ii) *C. rotundata* to monitor Zn contents in seawater of tropical seagrass bed.

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