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Different organs of *Enhalus acoroides* (Hydrocharitaceae) can serve as specific bioindicators for sediment contaminated with different heavy metals

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ABSTRACT

The tropical seagrass *Enhalus acoroides* is considered as a potential bioindicator for heavy metals. To date, studies on the accumulation of heavy metals in each specific seagrass organ have been limited. In this study, surface sediment and *E. acoroides* samples collected at six distinct beds from the coast of Khanh Hoa province, Vietnam, were evaluated for their heavy metal concentrations (Cd, Cu, Pb and Zn) to determine which organs of *E. acoroides* could be used as bioindicators for heavy metals. The metal concentrations in both the sediment and the seagrass organs were measured using inductively coupled plasma mass spectrometry (ICP-MS). Bio-concentration factor (*BCF*) and Metal pollution index (*MPI*) were determined on the seagrass organs, and Pearson's correlation and ANOVA were used for statistical analysis. The results showed that the highest *BCF* values of Cd and Zn were found in leaves, the highest *BCF* value of Cu was found in the rhizomes and the highest *BCF* value of Pb was found in the roots. Generally, *MPI* was lower in the sediment than in seagrass organs at the same locations. For Cd, Pearson's correlation showed that a significant positive correlation was only observed between sediment and leaves with respect to *BCF*. A significant positive correlation of *BCF* for Cu was observed between sediment and rhizome while significant positive correlations of *BCF* for Cu, Pb and Zn were observed between sediment and roots. Based on the results, it can be concluded that *E. acoroides* leaves should be used as a bioindicator for Cd and Zn, rhizomes for Cu, and roots for the three metals Cu, Pb and Zn.

1. Introduction

Heavy metals are predominantly released into the environment through anthropogenic activities and farming, and then accumulate in sediment with different levels (Gullstroem, 2006). These heavy metals have contaminated large areas of land in high concentrations due to sludge, the use of fertilizers and residues from metalliferous mines and smelting industries. Contaminated soils and sediments generate highly toxic substrates for plants (Goh and Chou, 1997; Halim et al., 2003). Coastal areas receive huge amounts of pollutants and heavy metals are a substantial component of such pollution (Govindasamy et al., 2011). Both essential and non-essential transition metal ions may be toxic to cells with the degree of toxicity depending on the ion concentration (Papenbrock, 2012). Excess Cu can cause chlorosis, inhibition of root growth and damage to plasma membrane permeability and may lead to ion leakage (De Vos et al., 1991). The leaf size and growth rate of the seagrass *Halophila ovalis* (R.Br.) Hook. *f.* were reduced when the ambient environment was contaminated by Cu and Pb (Ambo-Rappe et al., 2011). On the other hand, seagrasses tolerate high concentrations of heavy metals by sequestering them into the structural components of the leaf organ, thereby avoiding the negative effects on the more sensitive metabolic process in the cytoplasm (Ward, 1989).

Recently, it was demonstrated that the assessment of heavy metal concentration in coastal waters can be conducted by using halophytes such as marine algae (Fytianos et al., 1999; Chakraborty et al., 2014), seagrasses (Thangaradjou et al., 2010; Sudharsan et al., 2012; Ahmad et al., 2015), mangroves (Einollahipeer et al., 2013; Anouti, 2014) and other salt-tolerant plant species (Ghnaya et al., 2005) due to their high accumulation capacity. Among them, seagrasses were considered as potential bioindicators of heavy metals for coastal environments. Seagrasses are marine angiosperms and were adapted during evolution to aquatic life in tropical as well as in temperate regions (den Hartog, 1970). Seagrasses play important roles in coastal ecosystems (Short et al., 2007), acting as ecological engineers, and are important contributors to the primary production of the coastal area (Thangaradjou et al., 2014). Additionally, they have the ability to take up metals and other essential elements in two ways, from water

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through leaf surfaces and from the sediment through their roots and rhizomes (Llagostera et al., 2011).

In the Khanh Hoa province, Vietnam, in addition to a high population (approximately 500,000 persons in four cities and towns) (Statistics Office, Khanh Hoa, 2011) many industrial activities including shipyards, sugar plants and seafood processing companies are concentrated along the coast. Rapid urbanization as well as industrialization in coastal areas have increased since 2000s. Heavy metals are being continuously released to the marine environment from various sources, including the discharge of domestic sewage and industrial wastewater (Le et al., 2006). Previous studies have focused on the specific locations with sources of heavy metals, such as shipyards in the northern part of the province (Dung et al., 2014), or aquaculture at Nha Trang Bay (Ngo et al., 2009). The coastal zone in the Khanh Hoa province consists of several bays and lagoons including Van Phong Bay, Nha Trang Bay, Cam Ranh Bay and Thuy Trieu lagoon, where seagrass beds occur in an area of 1682 ha (Nguyen, 2010). Hence, more locations, especially in the southern parts with different heavy metal polluting sources, were investigated in this study.

Enhalus acoroides (Hydrocharitaceae) is widespread in the Indo-Pacific, Indian Ocean and it ranges from the southern Red Sea to northern Mozambique (Short and Waycott, 2010). Heavy metals in *E. acoroides* have been already been reported in several studies. In most studies the whole plant (consisting of leaves, rhizomes and roots) was used as bioindicator for heavy metals (Thangaradjou et al., 2010; Suwandana et al., 2011; Ahmad et al., 2015). In addition, above ground (leaves) and below ground material (including rhizomes and roots) showed significant differences in their accumulation of specific heavy metals (Li and Huang, 2012). *E. acoroides* can easily be divided into three main organs, namely leaves, rhizomes and roots. This raises the question of whether there are significant differences of the accumulation of different heavy metals in the three different organs of the tropical seagrass species *E. acoroides*. In order to use *E. acoroides* as a bioindicator for different heavy metals it is thus necessary to determine the most suitable plant organs that can be recommended for application.

2. Materials and methods

2.1. Sampling and pre-treatment

Seagrass (*E. acoroides*) and its rhizosphere sediment samples were collected from the Khanh Hoa coast at the following six locations: Tuan Le (TL, 109°21′; 12°45′), My Giang (MG, 109°17′; 12°29′), Song Lo (SL, 109°12′; 12°09′), Thuy Trieu Lagoon (TT, 109°11′; 12°02′), Cam Phuc Nam (CPN, 109°11′; 11°56′) and Cam Thinh (CT, 109°09′; 11°53′). Located in the northern part of the province, TL is within a mangrove forest and there are no industrial activities nearby. Shipyards are located at MG. TT is located in the southern part of the province, with several industrial activities such as sugarcane industry and concrete-mixing factories. CPN and CT were close to lobster culture sites and ports. Hence, TL was chosen as the control site. Sample collection was done during June 2015. Environmental conditions at all the locations are very similar, with a water temperature of 29.3–31 °C, a pH in the range 7.4–7.6 and a salinity of 31–32‰ (Nguyen et al., 2017). The depth of the seagrass meadows is about 1–1.5 m for all locations. At each location, five distinct plants were collected. From each plant, the healthy mature leaves (above ground), the rhizomes and the roots (below ground) were collected. The seagrass samples were washed with seawater to remove sediment and epiphytes that commonly attach to leaves, rhizomes and roots. Distinct organs (leaves, rhizomes and roots) were placed in separate polyethylene bags and transported to the laboratory, keeping them in an icebox. In the rhizosphere of each sample plant, a sample of the top 5 cm of sediment (approximately 500 g) was collected in acid-washed polyethylene bags using a PVC spade. Collection of seagrasses and sediment was carried out by SCUBA diving. Additionally, to establish background concentrations, one sediment sample at a depth of 150 cm was collected at a mangrove area connected to the sea at Nha Phu lagoon (NP), as suggested by Dung et al. (2014). The sealed sediment bags were kept in an icebox until they were transported to the laboratory for further analysis and longer term storage at −20 °C.

2.2. Sample analysis

For the sediment analysis, sediment samples were dried at 105 °C, ground, homogenized, and sieved. A 1 g quantity of dry sediment samples was used for digestion following the protocol of Stanković et al. (2015). The samples were placed into a Teflon digestion vessel (CEMAnalytical Matthews, NC, USA). After adding 10 mL of 1:1 (v/v) HNO_3 (65%, Merck, Darmstadt, Germany) into each vessel, they were sealed and placed into a microwave reaction system (MARS, CEMAnalytical Matthews, NC, USA). The samples were heated to 130 °C for 60 min and cooled for 10 min. Then 5 mL 30% H_2O_2 (Merck) was added and the mixture was heated again to 130 °C for 60 min for complete digestion. After cooling, the digested samples were filtered with filter papers (Whatman No. 1441-110, Sigma-Aldrich, Darmstadt, Germany) which were rinsed by 5% HNO_3 to release the residues in the digested samples. The samples were then make up to 100 mL with de-ionized water (Merck). The concentrations of the metals including cadmium (Cd), copper (Cu), lead (Pb) and zinc (Zn) were determined by inductively coupled plasma mass spectrometry (ICP-MS) (model 7700, Agilent 7700, Santa Clara, CA, USA). Briefly, the samples were introduced by a peristaltic pump with a speed of 0.1 rounds per second. In addition, blank and duplicate samples were included in the analytical procedure at random. Average values were used for the analysis. The ICP-MS was located in a temperature-controlled laboratory (22 °C) and was stabilized for a sufficient period of time (approximately 1 h before the measurements were started). The following instrumental parameters of ICP-MS were used: i) plasma gas flow: 15 L/min; ii) carrier gas flow: 1.0 L/min; iii) auxiliary gas flow: 1.5 L/min; iv) RF power: 1550 W; v) full quart; and vi) measuring mode: helium. For seagrass samples, green leaves, rhizomes and roots were homogenized in small mortars with pestles in liquid nitrogen. For the seagrass analysis, 0.1 g of each organ of each plant was analyzed in the same manner as the sediment samples. In order to evaluate the quality of the analytical method, a certified reference material (International Atomic Energy Agency-IAEA, 158, for marine sediment) was used for comparison (Campbell et al., 2008). The results were in agreement with the certified values, showing a good recovery rate (Table 1).

2.3. Pollution indices

Bio-concentration factor (*BCF*) suggested by Lewis et al. (2007) was used in this study. For each seagrass plant, the BCF was determined for each of the three organs as defined in Eq. (1) below. Metal pollution index (*MPI*) developed by Usero et al. (2005) was also used in this study. For each seagrass plant, the *MPI* was determined for each of the three organs and for the sediment. *MPI* is a measure of total metal

Table 1
Comparison between the measured concentrations and the certified data in the certified reference materials of International Atomic Energy Agency (IAEA) 158, for marine sediment (mean ± SD, n = 5).

Elements	IAEA 158 (marine sediment)		
	Certified (μg g^{-1})	Measured (μg g^{-1})	Recovery rate (%)
Cd	0.37 ± 0.09	0.43 ± 0.05	116
Cu	47.9 ± 5.27	50.0 ± 3.2	104
Pb	38.0 ± 7.7	36.8 ± 2.5	96
Zn	138 ± 13.2	134 ± 5.9	97

content and of interest is any systematic difference between speciation (sediment, root, rhizome, leaf) and between locations. *MPI* is defined in Eq. (2).

$$BCF = \frac{C_a}{C_x} \quad (1)$$

where C_a is the metal concentration in the seagrass organ in question (μg g^{-1} dry weight (wt)) and C_x is the metal concentration in the sediment (μg g^{-1} dry wt).

$$MPI = (C_1 \times C_2 \times \ldots \times C_n)^{\frac{1}{n}} \quad (2)$$

where C_n is the concentration of the metal *n* in the seagrass organ or the sediment, as required, and *n* is the total number of metals.

2.4. Statistical analysis

The difference of each heavy metal in the sediment collected at six locations was analyzed by single ANOVA. The difference of each metal in three distinct organs collected at six different locations was tested by two-way ANOVA. Correlation (correlation coefficient, *r*) between metal concentration in seagrass organs and rhizosphere sediment at six locations was performed by Pearson's correlation. Again, two-way ANOVA was used to analyze the differences of *BCF* among plant organs and locations, respectively. For the *MPI* values in sediment and seagrass organs, two-way ANOVA was also used to test the difference of the speciation (sediment, leaf, rhizome and root) collected in six distinct locations. The commercial statistics software package SPSS (version 19.0) for Windows was used for the statistical analysis. The chart was processed by Minitab™, version 2014 (State College, PA, USA).

3. Results

3.1. Metals in the rhizosphere sediment and in distinct organs

Three of the four heavy metal concentrations determined in the rhizosphere sediment, namely Cd, Pb and Zn, were lower than those given in the *TEL* guideline (CCME, Canadian Council of Ministers of the Environment, 2002) for all locations, while Cu concentration at MG was twice as high as given in *TEL* (Fig. 1). In general, Cu and Zn concentrations of the surface sediment of seagrass meadows near the shipyard factory were higher than at other locations, whereas the Pb concentration of surface sediment of seagrass meadows near the sugarcane factory was higher than at other locations. Higher concentrations of Cd were detected in the leaves compared to the rhizomes and roots. Hence, the levels of Cd concentration in *E. acoroides* were in the following order: leaf > root > rhizome (Fig. 2A).

The highest Cu values were determined in the rhizomes in comparison to the leaves and the roots (Fig. 2B). The range of Cu contents in *E. acoroides* was in the following order: rhizome > leaf > root. In contrast to Cu and Cd, the Pb concentration was higher in the roots than in the leaves and rhizomes (Fig. 2C). There was a consistent higher Zn concentration in the leaves than in the rhizomes and roots (Fig. 2D). The results of two-way ANOVA showed significant differences of the four metals between locations as well as between the three organs (Supplementary data 1).

3.2. Relationships of metal contents in seagrass organs and rhizosphere sediment

For Cd, a significant positive correlation was only observed between sediment and leaves. In contrast, significant positive correlations of Cu were found between sediment and rhizomes, and between sediment and roots. For Pb, there was a significant positive correlation between sediment and roots. Significant positive correlations of Zn were shown between sediment and roots and leaves (Table 2).

3.3. Bio-concentration factor and Metal pollution index

There were high ranking of *BCF* values in each organ and locations. The average *BCF* values of Cd in leaves were higher than for rhizomes and roots. In contrast, the average *BCF* value of Cu was higher for rhizomes than for leaves and roots. The average *BCF* values of Pb were higher in roots than in leaves and rhizomes. For Zn, the average *BCF* revealed large differences among three organs (Table 2, values in parentheses). The two-way ANOVA showed significant differences among locations as well as among three organs (Supplementary data 2).

The *MPIs* were calculated for the sediment and seagrass organs. *MPI* values of sediment at MG and TT were higher than the *MPI* values of the three organs. In contrast, *MPI* values of the sediment at CT and TL were lower than the *MPI* values of three organs. At CPN, *MPI* values of the

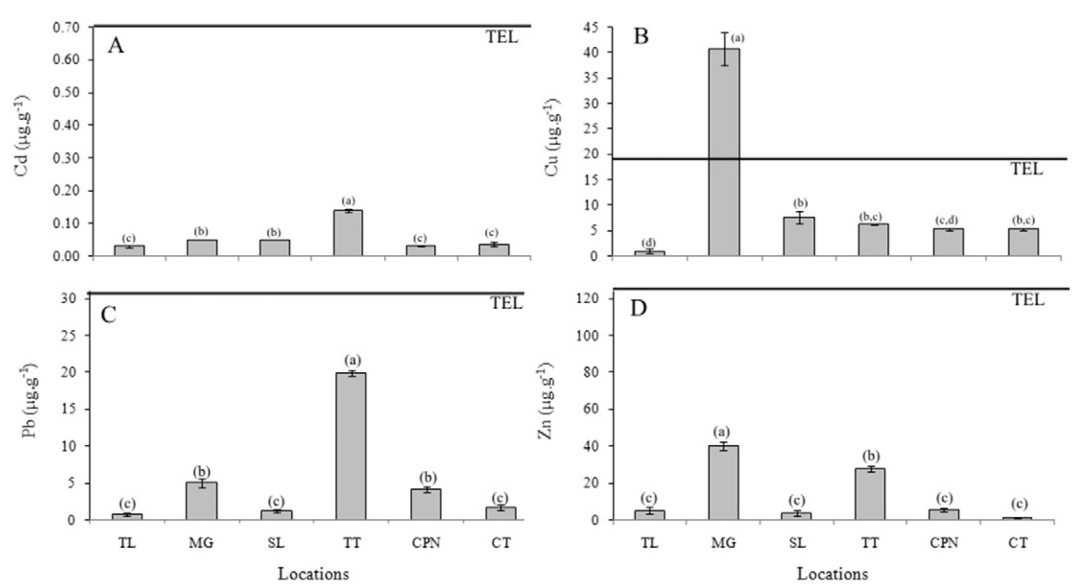

Fig. 1. The metal concentrations of A) Cd, B) Cu, C) Pb and D) Zn in the rhizosphere sediment at six seagrass beds. TL = Tuan Le, MG = My Giang, SL = Song Lo, TT = Thuy Trieu, CPN = Cam Phuc Nam, CT = Cam Thinh. TEL = Canadian sediment quality guidelines. Different letters (a–d) printed within the same column show significantly different means of observed data ($p < 0.01$) according to post hoc Tukey's HSD test. Data are presented in mean ± SD (n = 5).

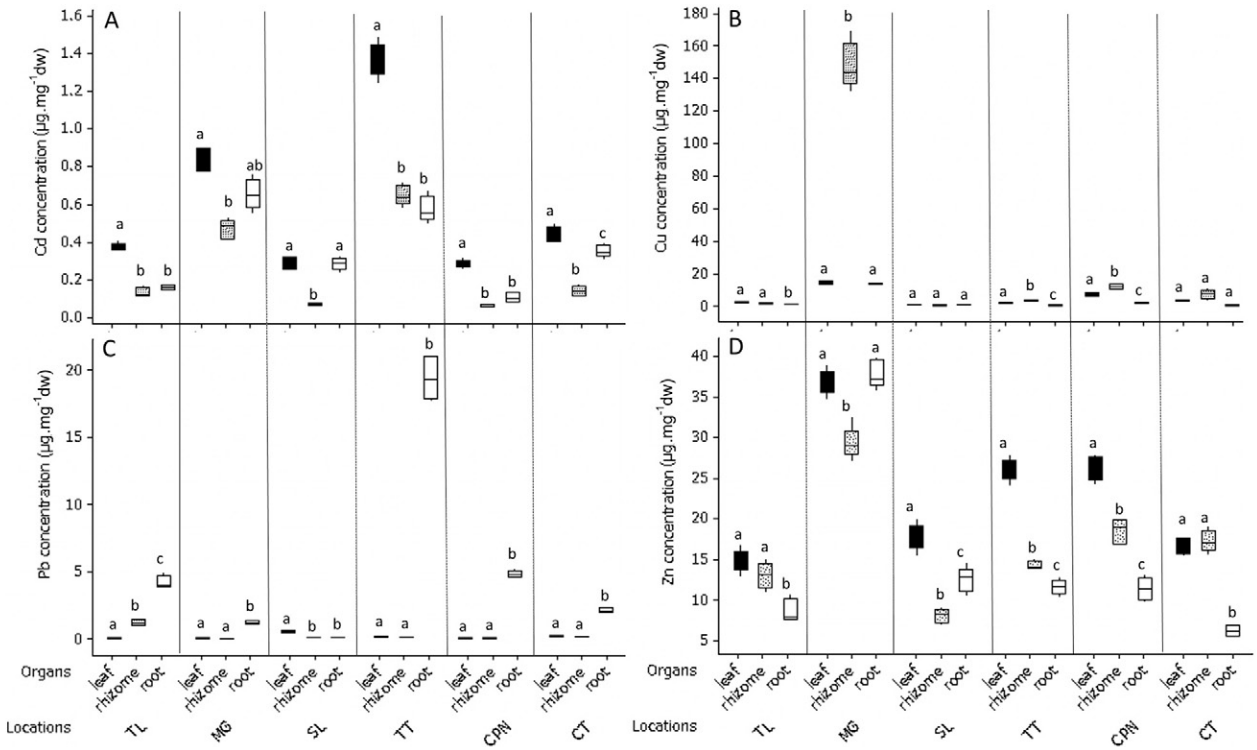

Fig. 2. The metal concentration of A) Cd, B) Cu, C) Pb and D) Zn in each organ of *E. acoroides*. Different letters (a–c) printed within the same column show significantly different means of observed data ($p < 0.01$) according to post hoc Tukey's HSD test. Data are presented in mean ± SD (n = 5).

sediment were higher than *MPI* values of rhizomes, but lower than *MPI* values of leaves and roots. At SL, the *MPI* value of the sediment was higher than *MPI* values of rhizomes and roots, but it was lower than *MPI* values of leaves (Fig. 3). Results of two-way ANOVA indicated that a significant difference was found among sediments, leaves, rhizomes and roots, and among the locations (Supplementary data 3). Correlations between *MPI* values and heavy metals of each organ are presented in Table 3. For the leaves and rhizomes, significant correlations were

Table 2
Correlation and *BCF* (values in parentheses) between metal concentration in seagrass organs and rhizosphere sediment at six sites. **Bold** indicates strong correlation. *: significance level ≤ 0.05, **: significance level ≤ 0.01. Data presented in mean ± SD, n = 5.

Speciation		TL	MG	SL	TT	CPN	CT
Cd							
Sediment/leaf	*r*	0.76 (12.1 ± 1.1)	**0.88** (16.7 ± 1.5)	**0.94** (5.8 ± 0.7)	**0.97** (9.8 ± 0.8)	0.61 (9.5 ± 1.1)	**0.99** (11.6 ± 1.2)
	Sig.	0.14	0.05*	0.02*	0.05*	0.28	0.01**
Sediment/rhizome	*r*	0.55 (4.4 ± 0.6)	0.20 (9.4 ± 1.3)	−0.52	−0.31	−0.25	−0.19
	Sig.	0.34	0.75	0.37	0.61	0.69	0.77
Sediment/root	*r*	−0.42	−0.27	−0.48	−0.04	0.76 (3.7 ± 1.2)	0.44 (9.3 ± 1.3)
	Sig.	0.48	0.66	0.41	0.95	0.14	0.46
Cu							
Sediment/leaf	*r*	0.36 (2.6 ± 1.3)	0.08 (0.3 ± 0.04)	−0.06	0.64 (0.3 ± 0.07)	0.23 (2.5 ± 0.4)	0.38 (0.7 ± 0.06)
	Sig.	0.55	0.91	0.93	0.25	0.71	0.53
Sediment/rhizome	*r*	**0.92** (1.5 ± 0.3)	**0.88** (3.5 ± 0.5)	0.84 (0.1 ± 0.03)	0.85 (0.6 ± 0.08)	**0.93** (4.4 ± 0.5)	**0.96** (1.4 ± 0.5)
	Sig.	0.03	0.05	0.08	0.07	0.02	0.01
Sediment/root	*r*	0.60 (0.9 ± 0.3)	0.80 (1.0 ± 0.2)	**0.95** (0.1 ± 0.02)	**0.91** (0.1 ± 0.02)	**0.89** (0.8 ± 0.1)	**0.79** (0.2 ± 0.05)
	Sig.	0.29	0.10	0.01	0.03	0.04	0.12
Pb							
Sediment/leaf	*r*	−0.15	0.07 (0.02)	0.26 (0.5 ± 0.05)	0.36 (<0.01)	0.08 (0.02)	0.32 (0.05)
	Sig.	0.81	0.92	0.67	0.55	0.90	0.60
Sediment/rhizome	*r*	0.87 (1.6 ± 0.5)	−0.38	0.11 (0.1 ± 0.01)	−0.31	−0.53	−0.27
	Sig.	0.05	0.52	0.86	0.62	0.37	0.67
Sediment/root	*r*	0.84 (5.6 ± 1.9)	**0.89** (0.2 ± 0.03)	0.84 (0.1 ± 0.03)	**0.91** (1.0 ± 0.12)	**0.89** (1.2 ± 0.1)	**0.92** (1.2 ± 0.2)
	Sig.	0.077	0.04*	0.07	0.03*	0.04*	0.03*
Zn							
Sediment/leaf	*r*	0.70 (3.1 ± 0.9)	**0.89** (1.0 ± 0.01)	**0.90** (4.8 ± 1.2)	0.82 (0.9 ± 0.1)	**0.94** (4.8 ± 0.6)	**0.93** (11.4 ± 2.2)
	Sig.	0.19	0.04	0.04	0.09	0.02*	0.23*
Sediment/rhizome	*r*	0.82 (2.7 ± 0.8)	0.43 (0.8 ± 0.05)	0.87(2.2 ± 0.5)	0.47 (0.5 ± 0.04)	0.5 1(3.4 ± 0.5)	0.87 (11.9 ± 2.1)
	Sig.	0.09	0.47	0.06	0.42	0.38	0.06
Sediment/root	*r*	**0.89** (1.9 ± 0.8)	**0.90** (1.0 ± 0.04)	**0.88** (3.4 ± 1.0)	**0.88** (0.4 ± 0.05)	0.79 (2.1 ± 0.3)	0.39 (4.3 ± 0.9)
	Sig.	0.05*	0.04*	0.05*	0.05*	0.11	0.52

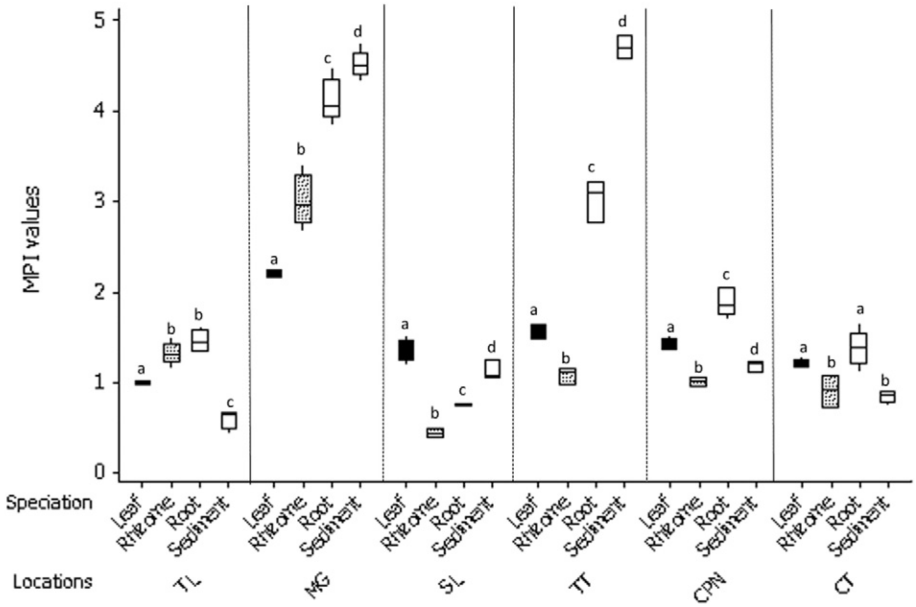

Fig. 3. *MPI* values of surface sediment and seagrass organs. Different letters (a–d) printed within the same column show significantly different means of observed data ($p < 0.01$) according to post hoc Tukey's HSD test. Data are presented in mean ± SD (n = 5).

found between *MPI* and three metals including Cd, Cu and Zn while in the root organs Cu, Pb and Zn showed significant correlations with *MPI* values.

4. Discussion

The *MPI* values from sediment indicated that TL was the least polluted location while at MG and TT higher heavy metal pollution was found. The total daily deposition measured at MG with sediment traps is very high (266.5 g DW m^{-2} day^{-1}) (Gacia et al., 2003). Another explanation might be the use of Cu slag as an abrasive material for removing rust from the surface of the ships at the shipyard company nearby (Dung et al., 2014). At TT, several factories are located along two sides of the lagoon such as the sugar cane industry and concrete-mixing factories. The sugar industry wastewaters are the main source of Pb pollution (Sudharka and Damodharam, 2015). In the marine environment, the main sources of Pb are from sewage inputs due to anthropogenic activities (Tchounwou et al., 2012; Thangaradjou et al., 2014).

For the leaves, the order of heavy metal concentration is Zn > Cu > Pb > Cd, which was also shown for *Cymodocea nodosa* (Bonanno and Di Martino, 2016). Results from the present study show that the Cd concentration in leaves was higher than in the rhizomes and roots. The higher Cd concentration in leaves than in other organs was found to be generally comparable to previous studies (e.g. Thangaradjou et al., 2010; Suwandana et al., 2011). In addition, a significant positive correlation was observed between sediment and leaves for this metal. Significantly

Table 3
Correlation between *MPI* values and concentration of heavy metals in each organ.

		Cd	Cu	Pb	Zn
Leaf					
MPI value of leaf organ	*r*	0.5	0.74	−0.1	0.94
	Sig.	0.05	<0.01	0.62	<0.01
Rhizome					
MPI value of rhizome organ	*r*	0.48	0.94	0.01	0.94
	Sig.	<0.01	<0.01	0.96	<0.01
Root					
MPI value of root organ	*r*	0.37	0.77	0.78	0.79
	Sig.	0.07	<0.01	<0.01	<0.01

different Cd concentrations among organs and the *BCF* values indicated that Cd was usually taken up by seagrass organs through physical sorption on the cell-surface and intracellular accumulation (Llagostera et al., 2011). The results from present study showed that the translocation and accumulation of Cd from the sediment to seagrass organs may occur mainly in the leaves. The leaves of *Zostera japonica* Asch. & Graebn. also accumulated the highest Cd concentration in comparison to below ground organs (Lin et al., 2016). Results from the present study have similar trend to those data reported for *Posidonia oceanica* (Di Leo et al., 2013). For *Zostera marina*, the Cd uptake occurs mainly via the leaves through passive absorption, which depends on the leaf surface, and the translocation of Cd between rhizome-roots and leaves occurred in both directions (Brinkhuis et al., 1980; Ward, 1989). The Cd concentration in the leaves was lower than in the root-rhizome organ of *Heterozostera tasmania* when plants were exposed to Cd in laboratory experiments (Fabris et al., 1982). For a better understanding of uptake, translocation, accumulation and detoxification of Cd in the seagrass *E. acoroides* similar laboratory experiments need to be carried out. Hence, leaves should be used as bioindicator for the Cd of the sediment.

Cu is essential for the growth and metabolic processes of plants and its accumulation is affected by processes of metabolic regulation (Yruela, 2009). For Cu, the results of the present study show lower concentration in the leaf organs than in rhizomes and roots. The results are in agreement with previously reported results for *Zostera noltii* Hornem (Wasserman and Wasserman, 2002). However, Cu concentrations in the leaves of *Cymodocea serrulata* (R.Br.) Asch. & Magnus and *Syringodium isoetifolium* (Asch.) Dandy were higher than in the below ground organs (Govindasamy et al., 2011). There was no correlation of their concentration between leaves and sediment. Very low *BCF* values between leaf and sediment (*BCF* < 1 at three of six locations) were found. The result of ANOVA also indicated that there was no significant difference of Cu concentration among the three organs in general. Therefore, leaves are not a suitable indicator for Cu in the environment.

Pb is not an essential element for plants and excessive amounts can cause growth inhibition and even death (Burkholder et al., 2007). In the present study, there was no significant correlation between Pb concentration of leaves and sediment. Moreover, the very low *BCF* values (<0.1) indicated that the leaf is not the main sink for the metal Pb. The accumulation characteristics are similar to those of Cu; thus, *E. acoroides* leaves are not suitable as an indicatior for Pb in the environment.

Zn, one of the essential elements for the growth and metabolic processes of plants, is needed by almost all plants in small quantities (Yamasaki et al., 2008). In this study, higher Zn concentrations were found in leaves than in the other organs. The results from the present study also support previous studies of the same species performed in Hainan Island (Li and Huang, 2012) and of other seagrass species like *Halophila stipulacea* (Forssk.) Asch. (Maleaa et al., 1995) and *P. oceanica* (Sanz-Lázaro et al., 2012). Also the Zn concentration showed a significant correlation between surface sediment and leaves. Zn is easily taken up by both roots and leaves of *Zostera capricorni* and transported to areas of active growth, but translocation between below and above ground organs is minimal (Howley et al., 2004). For another member of the Hydrocharitaceae, *Thalassia testudinum*, Zn dissolved in seawater was concentrated in the leaves, but little translocation to below ground organs occurred (Schroeder and Thorhaug, 1980). In addition, the higher *BCF* values and the significant difference of Zn concentration among organs indicated that *E. acoroides* accumulated higher Zn concentrations in the leaves than in the other organs. Therefore, it is recommended that leaves should be analyzed as a bioindicator for Zn in the environment. The *MPI* values showed significant correlations with the concentrations in the leaf for the three elements Cd, Cu and Zn. Hence, the combination of *BCF* values and a strong positive correlation of each metal and the *MPI* values suggest that the leaves of *E. acoroides* should be used as a bioindicator for both Cd and Zn.

E. acoroides can live up to ten years with a very slow rhizome growth, elongated at a rate of 5 cm per year (Vermaat et al., 1995). In general, the results from the present study show that the Cd and Pb concentrations in the rhizome were lower than for other organs. In addition, no significant correlations of Cd/Pb concentration between rhizome and sediment indicate that this organ is not suitable to be used as a bioindicator for Cd and Pb in the environment. In contrast to Cd and Pb, the Cu concentration in the rhizomes was higher than in the leaf organ. The higher Cu concentrations in rhizomes and roots compared to leaves are also known from *Halophila minor* (Zoll.) Hartog, another member of the Hydrocharitaceae, and members of the Cymodoceaceae, such as *Halodule pinifolia* (Miki) Hartog (Besar et al., 2008). The explanation for the highest Cu accumulation in the rhizome organ might be that Cu concentration in the surface sediment can be considered as extremely contaminated in the sediments sampled during this study and a previous study (Dung et al., 2014). For the other locations, no significant variation of the Cu concentration was observed between leaf and rhizome. *Cymodocea nodosa* showed a similar trend in the Cu accumulation in the two organs, leaf and rhizome (Llagostera et al., 2011). However, for *P. oceanica*, there was not much difference of Cu concentration in leaf and rhizome (Nicolaidou and Nott, 1998). In this study, significant correlations of Cu between surface sediment and rhizome and root organs were found. A similar trend was demonstrated for another member of the Hydrocharitaceae, *Thalassia testudinum* Banks & Sol. ex K.D.Koenig (Whelan et al., 2005). Cu and its chemical form are important in determining the pathway it will enter the plant compartment (Wasserman and Wasserman, 2002). Therefore, higher Cu concentration in rhizomes found in this study may also be due to its bioavailability from the sediment (Stanković et al., 2015). In addition, the higher *BCF* values of Cu also support the recommendation of using rhizomes as a bioindicator for Cu from the environment. Concentration of Zn in rhizomes was lower than in leaves and roots. The results from the present study indicate that there is no significant correlation of the Zn concentration between the rhizome and the sediment. This was different to *Cymodocea nodosa*, where a significant correlation of Zn between rhizomes and sediment was shown (Bonanno and Di Martino, 2016). The significant correlation of *MPI* values of rhizomes and Zn indicated that using the rhizomes as Zn bioindicator of the environment is not suitable.

The combination of the *BCF* values, the correlation and the *MPI* values revealed that the rhizomes are only suitable as a bioindicator of Cu among the four metals analyzed.

Generally, the Cd concentration in the root organ was lower than in the other organs. There was no significant correlation of the Cd concentration between the sediment and the root organ. However, there were significant correlations of Cu, Pb and Zn concentration between root organs and sediment in most cases. The significant correlations of Cu and Pb concentrations were found between root organs of *E. acoroides* and sediment (Werorilangi et al., 2016). Higher Pb concentrations in the roots than in other organs were also found in *Cymodocea nodosa* (Nicolaidou and Nott, 1998; Llagostera et al., 2011). Pb concentration in the root organ was higher than in the leaves and the rhizomes of *Thalassia hemprichii* (Ehrenb. ex Solms) Asch (Tupan et al., 2014). For land plants, the non-essential metals like Pb mainly accumulated in below ground organs (Wasserman and Wasserman, 2002; Ahmad et al., 2015). However, aquatic plants can absorb Pb from both seawater and sediment (Fritioff and Greger, 2006). Plants with numerous roots and root hairs like *E. acoroides* accumulate higher metal concentrations (Govindasamy et al., 2011). Although the *BCF* values of both Cu (<1, generally) and Pb (>1) in the roots were relatively low, the strong positive correlation of their concentration between roots and sediment support using this organ for indicating both of Cu and Pb in the environment. Roots are also considered as a potential organ for the indication of Zn due to the high *BCF* values and the strong correlation of its concentration between the root organ and the sediment. For *Cymodocea nodosa*, roots acted as bioindicators of several trace elements in sediments (Bonanno and Di Martino, 2016). The strong negative correlation of *MPI* values of this tissue and the three elements Cu, Pb and Zn also suggested that roots can be used as bioindicators for these.

5. Conclusion

The results of the present study suggest that leaves of *E. acoroides* can be used as a bioindicator for Cd and Zn, rhizomes for Cu, and roots for the three metals Cu, Pb and Zn. The study also paves the way to plan investigations on one of the subsequent research questions on the role of metal-binding compounds, such as phytochelatins, which are known to be involved in heavy metal detoxification.

There are major and significant differences among the accumulation levels of heavy metals in the different organs. To conclude, the differentiation into different organs and their analysis is highly recommendable for the use of *E. acoroides* as bioindicator. The second major progress and difference in comparison to the published data is that the present study was carried out at six different seagrass beds that contain significantly different levels of heavy metals. Therefore, the present study provides not only the experimental data basis for a bioindicator but also includes the proof of principle that the seagrass system is of high application value.

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Appendix A. Supplementary data

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