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Tropical Seagrass as a Bioindicator of Metal Accumulation

(Rumput Laut Tropika sebagai Penunjuk Biologi Pengumpulan Logam)

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ABSTRACT

Seven species of tropical seagrass found at seagrass bed located in Johor, Malaysia were analysed for As, Cu and Cd accumulation. The species were identified as Enhalus acoroides, Halophila minor, Halophila spinulosa, Halophila ovalis, Thalassia hemprichii, Halodule uninervis *and* Cymodocea serrulata. *Seagrass plant is rapidly becoming one of the methods to determine the overall health condition of aquatic environment. Each seagrass samples were collected and divided into three parts i.e roots, rhizomes and leaves. Samples were grinded, digested and the correlation between each part was analysed using SPSS version 16. Each part of seagrass tissues have the ability to assimilate metals for example the concentration of As, Cu and Cd in tropical seagrass were in the range of 5-48, 6-60 and 10-69 µg/gDW, respectively.* Halophila minor *and* Halophila ovalis *indicates positive correlations to translocate metals (As, Cu and Cd) in plants parts (leaves-rhizomes, rhizomes-roots and roots-leaves). Seagrass can accumulate metals depending on pollution that occur, seasonal variation and internal capabilities to translocate metals. The seagrass species especially* Halophila ovalis *and* Halophila minor *can act as bioindicator for metal pollution.*

Keywords: Bioindicator; metal contamination; seagrass

ABSTRAK

Tujuh spesies rumput laut tropika yang dijumpai di beting rumput laut yang terletak di Johor, Malaysia telah dianalisis untuk pengumpulan logam As, Cu dan Cd. Spesies yang dikenal pasti adalah Enhalus acoroides, Halophila minor, Halophila spinulosa, Halophila ovalis, Thalassia hemprichii, Halodule uninervis *dan* Cymodocea serrulata. *Rumput laut boleh dijadikan sebagai salah satu kaedah untuk menentukan keadaan kesihatan keseluruhan persekitaran akuatik. Setiap sampel rumput laut dikumpulkan dan dibahagikan kepada tiga bahagian iaitu akar, rizom dan daun. Sampel dikisar, dicerna dan korelasi antara setiap bahagian telah dianalisis menggunakan perisian SPSS versi 16. Setiap bahagian tisu rumput laut mempunyai keupayaan untuk mengasimilasikan logam contohnya keputusan menunjukkan kepekatan As berada dalam julat 5-48 µg/gDW, Cu dalam julat 6-60 µg/gDW dan Cd dalam julat 10-69 µg/gDW.* Halophila minor *dan* Halophila ovalis *menunjukkan korelasi positif untuk memindahkan logam (As, Cu dan Cd) pada tumbuhan (daun-rizom, akar rizom dan akar-daun). Rumput laut tropika boleh mengumpul logam bergantung kepada pencemaran yang berlaku, perbezaan musim dan keupayaan dalaman rumput laut untuk memindahkan logam. Spesies rumput laut terutama* Halophila ovalis *dan* Halophila minor *boleh dijadikan sebagai petunjuk biologi.*

Kata kunci: Pencemaran logam; penunjuk biologi; rumput laut

INTRODUCTION

Estuaries are exposed to anthropogenic contaminants including complex mixtures of heavy metals from industrial, agricultural and domestic waste directly via rivers or through atmospheric deposition (Emelogu et al. 2013; Gillet et al. 2008; Lafabrie et al. 2007; Villate et al. 2013). Human activities such as coastal land disturbance, motor boating and dredging increases pollutants (Burkholder et al. 2007; Lewis et al. 2007). One way to measure the degree of pollution is by using seagrass as a bioindicator for metal contamination (Ferrat et al. 2003; Prange & Dennison 2000; Schlacher-Hoenlinger & Schlacher 1998). Seagrass species are rooted angiosperms marine plants that are widely distributed in a large area known as seagrass bed (Bastyan & Cambridge 2008; Kirkman 1990; McKenzie et al. 2001; Vermaat et al. 2004). Seagrass bed plays an ecological role in coastal ecosystems as the most productive plant communities that support high biodiversity and food for a variety of marine organisms (Duarte & Chiscano 1999; Eklöf et al. 2008; Jackson et al. 2006; Lee et al. 2007; Waycott et al. 2005). Seagrass plant is rapidly becoming one of the method to determine the overall health and condition of aquatic environment (Bortone & Turpin 2000; Costantini et al. 1991; Macinnis-Ng & Ralph 2004). Seagrass have a remarkable metal bioaccumulation capacity since it interacts directly with both water column and pore water through the leaves and roots as ionic uptake hence it can reflect the overall health of coastal water (Llagostera et al. 2011). Seagrasses are protected under International Union for the Conservation of Nature Red list of Threatened Species and the biggest diversity of tropical seagrass was identified at Indo-Pacific region (Short et al. 2011, 2001). In addition, the seagrass bed serves as a 'nursery habitat' for the most productive plant communities that support food and shelter for a diverse biodiversity and vulnerable marine organisms (Duarte & Chiscano 1999; Heck & Valentine 2006, Jackson et al. 2006; Lee et al. 2007; Waycott et al. 2005). However, the continuously increasing pollution will cause the seagrasses to die-off once pollution reaches lethal levels and this intertidal habitat will be lost for good as there is little opportunity for the habitat itself to migrate (Hadley 2009).

Currently data recorded for metal accumulation in tropical seagrass in Southeast Asia is very limited (Ooi et al. 2011). Therefore, the aim of this study was to determine the capability of 7 tropical seagrass species (*Enhalus acoroides, Halophila minor, Halophila spinulosa, Halophila ovalis, Thalassia hemprichii, Halodule uninervis* and *Cymodocea serrulata*) at Pulai River Estuary to accumulate As, Cu and Cd. The seagrass bed at Pulai River Estuary is the largest in Malaysia with an approximate size area of 3.15 km^2. The estuary is listed as a Ramsar Site and receives water from the Straits of Malacca and nearby rivers (Rusli 2012). It is located nearby Malaysia - Singapore Second Link, Port of Tanjung Pelepas and Tanjung Bin Power Plant.

MATERIALS AND METHODS

SAMPLING

Seagrass samples were collected at Pulai River Estuary seagrass bed from July, 2011 to March, 2012. The samples complete with roots, rhizomes and leaves were manually collected at every 20 m interval on the seagrass bed using transect method. The starting sampling point on seagrass bed is at 1º 20' 22.53" N, 103º 36' 16.13" E. Pulai seagrass bed is an intertidal zones in which during high tide the depth from surface of seawater to seagrass bed is approximately 3.5 m. Figure 1 shows the location, perimeter, upper view and lateral view of Pulai seagrass bed. The slope gradients were gradually increasing and it can be visually seen like a big patch of grassland during low tide. The gradient shows a gradual slope at every edge of the seagrass bed. The size of the seagrass bed is 1.8 km in length by 0.1 km width. The distance between seagrass bed to Port of Tanjung Pelepas, Tanjung Bin Power Plant and Bunker Terminal are 3.7, 5.6 and 5.5 km, respectively.

The samples collected were later rinsed with seawater to remove sediment at roots and rhizomes. Then, the samples were packed in clean plastic bags, sealed, placed in a cool box containing ice, then transported to the laboratory and frozen at -20ºC prior to analysis (Sanchiz et al. 2000).

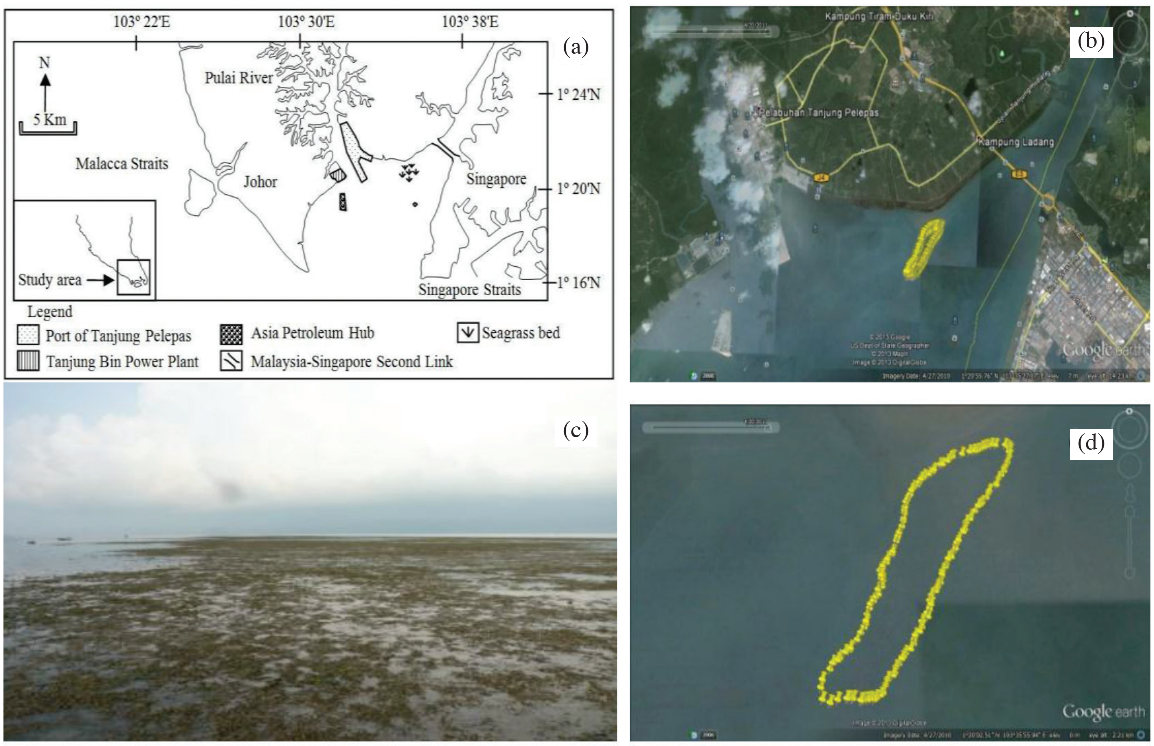

FIGURE 1. a) Location of the seagrass bed relative to the Port of Tanjung Pelepas, Tanjung Bin Power Plant and the Malaysia-Singapore Second link bridge, b) Pulai Estuary seagrass bed: the yellow-dotted irregular oval is the perimeter of the seagrass bed (goggle earth), c) lateral view of the seagrass bed on site during low tide, the gradient is gradually slope at the edge of seagrass bed and d) upper view of the seagrass bed (google earth)

SAMPLE PROCESSING AND DATA ANALYSIS

Each seagrass sample was collected and mixed at different locations on the seagrass bed. The sample was sorted into leave, rhizome and root. The seagrass samples were collected in a set of 12 specimens. Samples were dried at room temperature until it reaches a constant weight and grinded using agate mortar into homogenous powder (Llagostera et al. 2011). 0.3 g of the sample was later placed in Pyrex tubes with Teflon closure and added with 5 mL HNO_3, incubated for 1 h in a water bath at 100ºC, then cooled, filtered with 0.2 μm nylon membrane and diluted in 15 mL deionised water. Metal analysis was carried out using Perkin Elmer Atomic Absorption Spectrophotometer Model AAnalyst 400.

The Pearson's correlation coefficients of metals concentration between roots, rhizomes and leaves tissues were analysed using SPSS version 16 software. For easy references and recognition the seagrasses were given code names as shown in Table 1.

TABLE 1. Code names of seagrasses

Code name	Species of seagrass
Ea	*Enhalus acoroides*
Hm	*Halophila minor*
Hs	*Halophila spinulosa*
Ho	*Halophila ovalis*
Th	*Thalassia hemprichii*
Hu	*Halodule uninervis*
Cs	*Cymodocea serrulata*

RESULTS AND DISCUSSION

Figure 2 shows the concentration of As, Cu and Cd in different tissues of the 7 tropical seagrass. The results were expressed as μg of metal per gDW of analysed tissue where DW refers to dry weight. All seagrass species were able to accumulate As, Cu and Cd. The concentration of As, Cu and Cd in different tissues of the 7 tropical seagrass were in the range of 5-48, 6-60 and 10-69 μg/gDW, respectively.

At Pulai River Estuary, seagrass tissue can assimilates higher Cd content rather than Cu and As. Cd sources may come from Port of Tanjung Pelepas, Bunker Terminal and the Straits of Malacca which can cause the reclamation work at the estuary and increasing shipping activities. The Cd discharged and flow into the seawater will then uptake by the seagrass. Marine plants that accumulate Cd from seawater is considered as toxic at higher concentration (Ripperger et al. 2007).

Cu can also be accumulated in all the seven species of tropical seagrass. Bioavailability and toxicity of Cu to aquatic organisms depends on the total concentration at the estuary. Elevated Cu concentration in estuarine environments may results from the industrial and municipal waste, urban runoff, rivers and shorelines

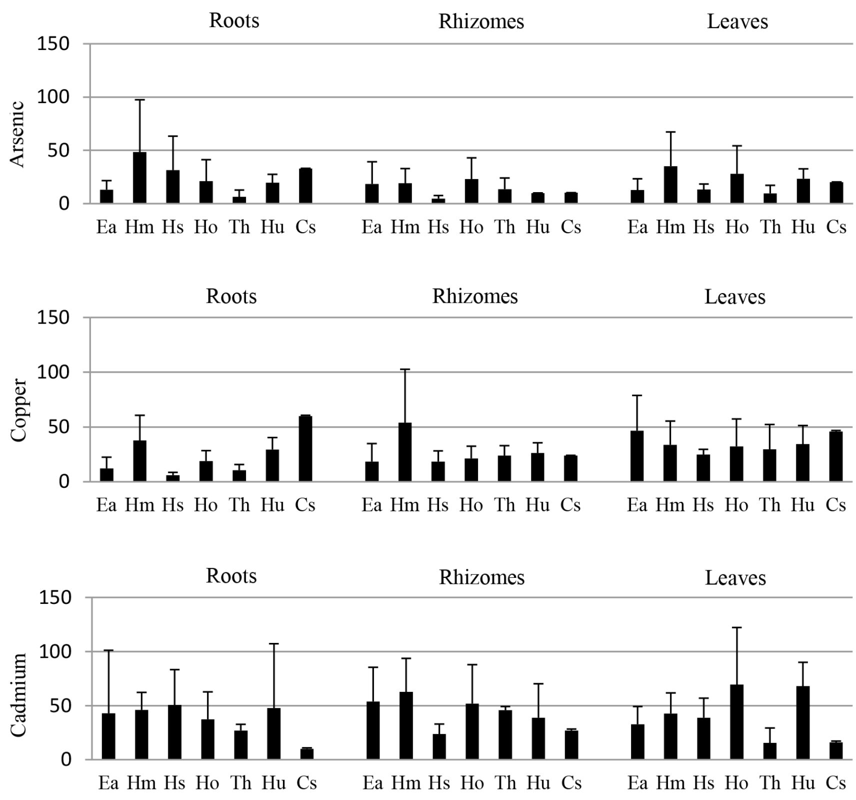

FIGURE 2. Concentration of As, Cu and Cd in seagrass tissues; all data express by μg gDW^{-1}; Mean and standard error are shown

erosion related to human activities from Pulai River. Cu is an essential component of many proteins, in excess it can cause toxicity and lead to damaging effect in marine plant tissue particularly to mechanisms involved in photosynthesis (Grant et al. 2003). In addition, previous study reported that Cu is toxic to many phytoplankton and zooplankton at high concentration (Vance et al. 2008).

Tropical seagrasses are able to assimilate As from seawater. The source of As could come from the biggest coal-fired in Southeast Asia known as Tanjung Bin Power Plant. As is toxic and has no biological role (Rensing & Rosen 2009). As appears to be elevated in marine biota because of its ability to accumulate As from seawater. Macropyhtes transform arsenate into non-volatile methylated As compounds such as methanearsonic and dimethlarsinic acids, methylation of As (unlike methylation of Hg) greatly reduced toxicity and is known as detoxification process (Eisler 2000).

Undesired metal contaminates (As, Cu and Cd) were trapped on seagrass bed due to the increasing gradient surface of sediment. All seagrass tissues could reasonably have a bioindicator role in reducing metals contamination that comes from seawater and sediment, therefore partially can reduce the effect of pollution on Pulai River Estuary. The pollutants are believed to come from nearby development, land use and shipping oil spills from the Malacca and Singapore Straits. The seagrass bed's ability to trap metal contaminants is evident that it is functioning well, as stated by Short and Coles (2001) where seagrass bed acts as a buffer zone. Aside from that, the seagrass bed cleanses seawater by absorbing dissolved metals and is a bioindicator that reflects the overall health of the aquatic ecosystem (Costantini et al. 1991; Eklöf et al. 2008; Kaldy 2006; Llagostera et al. 2011; Macinnis-Ng and Ralph 2004).

Table 2 shows the concentration of As, Cu and Cd from the previous studies for temperate and tropical seagrasses. In this paper, Cu and Cd accumulates higher concentration in *Cymodocea serrulata, Thalassia hemprichii, Enhalus acoroides, Halodule uninervis* and *Halophila ovalis* than reported in Flores Sea, Indonesia (Nienhuis 1986). The different value of accumulation is due to the different metals rate at surrounding area. Flores Sea is situated without industry and untouched isolated areas whereas Pulai River Estuary seagrass bed is surrounded by on-going development between Malaysia and Singapore. Metals concentrations in tropical seagrass are basically depend on the metals presence either the area is polluted or not.

The Cd concentrations reported by Kilminster (2013) were lower compared with this study. The concentrations were different because of seasonal variation. At Pulai River Estuary, the weather condition is always warm and the range of seawater temperature is 27.0±3.0ºC whereas Kilminster (2013) collected his samples in winter. As stated by Eisler (2000) Cd uptake will increase with the increasing seawater temperature in a range of 5 to 25ºC. Cd concentration in this study is similar to studies reported by Malea (1994) and Malea et al. (1994) where the seawater temperature is in the range of 11-24ºC.

The concentrations of As and Cu in the seagrass tissues are also similar to the results reported by previous studies (Eisler 2000; Kilminster 2013; Malea 1994; Malea et al. 1994; Schlacher-Hoenlinger & Schlacher 1998). Generally, bioavailability and toxicity of marine plants that accumulates As, Cu and Cd from seawater and sediment are dependent on its total metal concentrations in the estuary and seasonal variation (Eisler 2000; Malea et al. 1994). Seagrass tissues are considered toxic at higher metal concentration because it tends to accumulate metals from seawater and sediment to reduce metal contamination from the surroundings (Macinnis-Ng & Ralph 2002; Ripperger et al. 2007).

Table 3 shows the correlations of As in roots-rhizomes, rhizomes-leaves and leaves-roots, where there are significant correlations between different seagrass tissues for *Enhalus acoroides*, *Halophila minor*, *Halophila ovalis* and *Cymodocea serrulata*. There are significant correlations for accumulation of Cu between roots-rhizomes, rhizomes-leaves and leaves-roots in *Halophila minor*, *Halophila ovalis*, *Halodule uninervis* and *Cymodocea serrulata* (Table 4). As for Cd accumulation (Table 5), only *Halophila minor*, *Halophila ovalis* and *Cymodocea serrulata* were found to have significant correlations between roots-rhizomes, rhizomes-leaves and leaves-roots. Based on the correlation analysis, it can be summarized that in *Halophila minor*, *Halophila ovalis* and *Cymodocea serrulata* there are positive correlations between roots, rhizomes and leaves in metals (As, Cu and Cd). This indicate that *Halophila minor*, *Halophila ovalis* and *Cymodocea serrulata* have effective active uptake kinetics and internal tissues to translocate As, Cu and Cd to the entire plant system.

The correlation data (As, Cu and Cd) for leaves, rhizomes and roots are different because metal accumulation by seagrass are governed by multitudes of factors such as the relative metal availability for seagrass uptake, plant physiology including leaves may differ uptake kinetics and passive absorption from roots and internal translocation among plant parts, be it passively or actively (Llagostera et al. 2011). Active uptake is a movement and transported biochemical from lower concentration to higher concentration that requires energy known as uphill process whereas passive uptake known as downhill process are driven by mass flow (Zhan et al. 2010). There are two phases for absorbing metals for terrestrial plant, the first phase is the metals diffuse from the surrounding into the root; in the second phase metals gradually distributes and accumulates in plant tissues (Kvesitadze et al. 2009). In this study, seagrass tissues (roots, rhizomes and leaves) may absorb the metals simultaneously because the seagrass were submerged in seawater.

TABLE 2. Concentration of As, Cu and Cd from temperate and tropical seagrass species in µg gDW^{-1}

Seagrass		Location	Metals concentration			References
			Arsenic	Copper	Cadmium	
Temperate						
Posidonia oceanica	Whole plant	Mediterranean	-	-	5.38±0.14	Lafabrie et al. 2007
	Whole plant	Mediterranean	-	-	3.39±0.12	Lafabrie et al. 2007
	Whole plant	Mediterranean	-	-	1.70±0.06	Lafabrie et al. 2007
	Whole plant	Italy	-	14.0±17.0	0.9-1.3	Schlacher-Hoenlinger & Schlacher 1998
Halophila stipulacea	Whole plant	Greece	-	2.0-82.7	1.3-93.7	Malea 1994
	Root and rhizome	Greece	-	1.9-79.7	0.88-51.7	Malea 1994
	Leaf	Greece	-	1.9-81.3	2.5-85.7	Malea 1994
Posidonia oceanica	Leaf	Mediterranean	-	-	2.1-25.0	Sanchiz et al. 2000
	Rhizome	Mediterranean	-	-	0.6-2.0	Sanchiz et al. 2000
	Root	Mediterranean	-	-	0.9-2.2	Sanchiz et al. 2000
	Whole plant	Greece	-	2.8-148.0	2.7-44.0	Malea et al. 1994
Tropical						
Cymodocea rotundata	Leaf	Indonesia	-	6.6±1.6	0.49±0.24	Nieuhuis 1986
	Shoot	Indonesia	-	4.5±1.6	0.40±0.02	Nieuhuis 1986
	Rhizome and root	Indonesia	-	4.5±5.0	0.22±0.03	Nieuhuis 1986
Cymodocea serrulata	Leaf	Indonesia	-	5.8±2.2	0.68±0.15	Nieuhuis 1986
	Shoot	Indonesia	-	3.1±1.0	0.27±0.08	Nieuhuis 1986
	Rhizome and root	Indonesia	-	3.1±1.8	0.17±0.03	Nieuhuis 1986
Thalassia hemprichii	Leaf	Indonesia	-	7.0±2.3	0.57±0.33	Nieuhuis 1986
	Shoot	Indonesia	-	5.0±2.0	0.42±0.28	Nieuhuis 1986
	Rhizome and root	Indonesia	-	2.9±1.2	0.24±0.19	Nieuhuis 1986
Syringodium isoetifolium	Leaf	Indonesia	-	3.9±1.8	0.16±0.63	Nieuhuis 1986
	Shoot	Indonesia	-	4.1±1.7	0.21±0.08	Nieuhuis 1986
	Rhizome and root	Indonesia	-	6.8±8.1	0.16±0.05	Nieuhuis 1986
Enhalus acoroides	Leaf	Indonesia	-	4.4±2.3	0.36±0.19	Nieuhuis 1986
	Shoot	Indonesia	-	3.7±2.3	0.16±0.12	Nieuhuis 1986
	Rhizome and root	Indonesia	-	2.6±2.3	0.12±0.07	Nieuhuis 1986
Halodule uninervis	Leaf	Indonesia	-	5.6±2.4	0.60±0.19	Nieuhuis 1986
	Shoot	Indonesia	-	3.4±3.0	0.24±0.07	Nieuhuis 1986
	Rhizome and root	Indonesia	-	2.7±0.4	0.25±0.07	Nieuhuis 1986
Halodule pinifolia	Whole plant	Indonesia	-	6.7±5.2	0.36±0.21	Nieuhuis 1986
Thalassodendron ciliatum	Leaf	Indonesia	-	6.0±0.9	1.54±0.56	Nieuhuis 1986
	Shoot	Indonesia	-	3.3±0.9	0.98±0.20	Nieuhuis 1986
	Rhizome and root	Indonesia	-	3.4±0.7	0.73±0.39	Nieuhuis 1986
Halophila ovalis	Whole plant	Indonesia	-	17.3±15.1	0.41±0.12	Nieuhuis 1986
	Leaf	Australia	5.4-8.5	6.6-42.2	0.18-0.53	Kilminster 2013
	Rhizome	Australia	9.2-20.0	3.3-26.6	0.04-0.38	Kilminster 2013
	Shoot	Australia	15.4-35.0	11.1-80.0	0.05-0.38	Kilminster 2013

TABLE 3. Correlation analysis for As concentration in roots, rhizomes and leaves with r: correlation coefficient; p-value: significance level settled at 0.05 (2-tailed); *n*=12

	Roots-rhizomes		Rhizomes-leaves		Leaves-roots	
	r	p-value	r	p-value	r	p-value
Ea	0.92	0.00	0.98	0.00	0.98	0.00
Hm	0.99	0.00	1.00	0.00	1.00	0.00
Hs	0.90	0.01	0.55	0.20	0.86	0.01
Ho	0.98	0.00	1.00	0.00	0.98	0.00
Th	0.17	0.14	0.93	0.00	0.06	0.39
Hu	0.51	0.05	0.56	0.03	0.04	0.62
Cs	1.00	0.00	1.00	0.00	1.00	0.00

Table 4. Correlation analysis for Cu concentration in roots, rhizomes and leaves with r: correlation coefficient; p-value: significance level settled at 0.05 (2-tailed); *n*=12

	Roots-rhizomes		Rhizomes-leaves		Leaves-roots	
	r	p-value	r	p-value	r	p-value
Ea	0.67	0.10	0.72	0.07	0.99	0.00
Hm	0.98	0.00	1.00	0.00	0.97	0.00
Hs	0.02	0.96	0.82	0.02	0.88	0.00
Ho	1.00	0.00	0.86	0.01	0.87	0.01
Th	0.49	0.26	0.03	0.55	0.28	0.05
Hu	1.00	0.00	1.00	0.00	1.00	0.00
Cs	1.00	0.00	1.00	0.00	0.99	0.00

TABLE 5. Correlation analysis for Cd concentration in roots, rhizomes and leaves with r: correlation coefficient; p-value: significance level settled at 0.05 (2-tailed); *n*=12

	Roots-rhizomes		Rhizomes-leaves		Leaves-roots	
	r	p-value	r	p-value	r	p-value
Ea	0.94	0.01	0.16	0.20	0.71	0.00
Hm	0.99	0.00	1.00	0.00	0.99	0.00
Hs	0.40	0.03	0.58	0.23	0.12	0.27
Ho	0.99	0.00	1.00	0.00	1.00	0.00
Th	0.86	0.00	0.90	0.02	0.16	0.20
Hu	0.84	0.01	0.50	0.67	1.00	0.00
Cs	0.99	0.00	1.00	0.00	0.93	0.00

CONCLUSION

In selecting seagrass species as a bioindicator, it should be taken into consideration that the highest accumulation of As is observed in *Halophila minor* (roots), the highest accumulation of Cu is in *Halophila minor* (rhizomes) and the highest accumulation of Cd is in *Halophila ovalis* (leaves). *Halophila minor* and *Halophila ovalis* also indicates a positive correlation to translocation of metals among plants parts, therefore *Halophila minor* and *Halophila ovalis* can be useful as bioindicators.

Two areas of research are identified as important consequences of this study. The various tissue structures of the seagrasses ought to be examined in order to explain their different absorbtion potentials, also the lethal concentration level for different seagrasses and for different metals should be further analysed.

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