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Tape seagrass (*Enhalus acoroides*) as a bioindicator of trace metal contamination in Merambong shoal, Johor Strait, Malaysia

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ABSTRACT

Revealing the potential of seagrass as a bioindicator for metal pollution is important for assessing marine ecosystem health. Trace metal (^{111}Cd , ^{63}Cu , ^{60}Ni , ^{208}Pb , ^{66}Zn) concentrations in the various parts (root, rhizome, and blade) of tape seagrass (*Enhalus acoroides*) collected from Merambong shoal of Sungai Pulai estuary, Johor Strait, Malaysia were acid-extracted using a microwave digester and analysed via inductively coupled plasma-mass spectrometry (ICP-MS). The ranges of trace metal concentrations (in $\mu\text{g g}^{-1}$ dry weight) were as follows: Cd (0.05–0.81), Cu (1.62–27.85), Ni (1.89–9.35), Pb (0.69–4.16), and Zn (3.44–35.98). The translocation factor revealed that *E. acoroides* is a hyperaccumulator plant, as its blades can accumulate high concentrations of Cd, Cu, Ni, and Zn, but not Pb. The plant limits Pb mobility to minimize Pb's toxic impact. Thus, *E. acoroides* is a potential bioindicator of metal pollution by Cd, Cu, Ni, and Zn in estuarine environments.

Estuarine and marine ecosystems are exposed to an array of pollutants. Among these pollutants, trace metals have more prominent distribution, as they enter ecosystems via a range of point and nonpoint sources (Palma et al., 2015). These pollutants eventually settle down into the sediment and biota of the aquatic environment. Prolonged exposure to a high level of metal contaminants may lead to adverse effects on organisms, the aquatic environment, and human health (Landner and Reuther, 2005; Nordberg et al., 2014).

The degree of trace metal contamination in environmental matrices such as sediment (Zulkifli et al., 2010; Haris and Aris, 2015) and aquatic water (Looi et al., 2013) has been measured and reported in numerous studies that were designed to evaluate the environmental quality of marine ecosystems. However, these approaches did not contribute knowledge of the bioavailability of the elemental fractions nor ecotoxicology purposes and require periodic analyses (Fränzle, 2006). Marine organisms such as mussels (Chandurvelan et al., 2015), fish (Bashir et al., 2013), molluscs (Yap and Cheng, 2013), and algae (Chakraborty et al., 2014) are frequently used as bioindicators of trace metal contamination. The contamination in tissues of marine organisms can provide a time integrated measurement of bioavailable trace metals. It also could give early signs of any environmental disruption (Rainbow, 1995).

The marine angiosperm, seagrass, has been used as a bioindicator for metal monitoring purposes (Llagostera et al., 2011; Malea and Kevrekidis, 2013; Ahmad et al., 2015a; Bonanno and Di Martino, 2016)

owing to its high bioaccumulation capacity (Ahmad et al., 2015a). The blades and roots of seagrass interact directly with the water column and pore water as the whole plant is submerged. This feature allows seagrass to take in ions through their blades and roots (Romero et al., 2006). The concentration of trace metals in seagrass parts can reflect the metal contamination history of marine ecosystems (Lafabrie et al., 2007). Furthermore, seagrass is sensitive to natural and anthropogenic strain, long living, widely scattered, and easily identified and sampled (Reizopoulou and Nicolaidou, 2004).

Several studies on seagrass in relation to metal contamination have been conducted by numerous researchers from different regions of the world (Llagostera et al., 2011; Suwandana et al., 2011; Li and Huang, 2012; Kilminster, 2013; Ahmad et al., 2015a; Brito et al., 2016; Tuapattinaya et al., 2016). Yet, only a few notable studies on metal distribution in seagrass exist for Malaysia (Besar et al., 2008; Ahmad et al., 2015a). These studies focused on trace metal monitoring but did not investigate the translocation efficiency in different seagrass parts (root, rhizome, and blade). Therefore, the present study aims to (i) determine the trace metal contamination in various parts (root, rhizome, and blade) of *Enhalus acoroides*, commonly known as tape seagrass, from Merambong shoal, Johor Strait, Malaysia, and (ii) determine the capability of *E. acoroides* to act as a bioindicator based on the translocation factor.

Merambong shoal (1°20'14.53"N, 103°36'08.70"E) is located within the Sungai Pulai estuary, Johor Strait, Malaysia (Fig. 1). It is a

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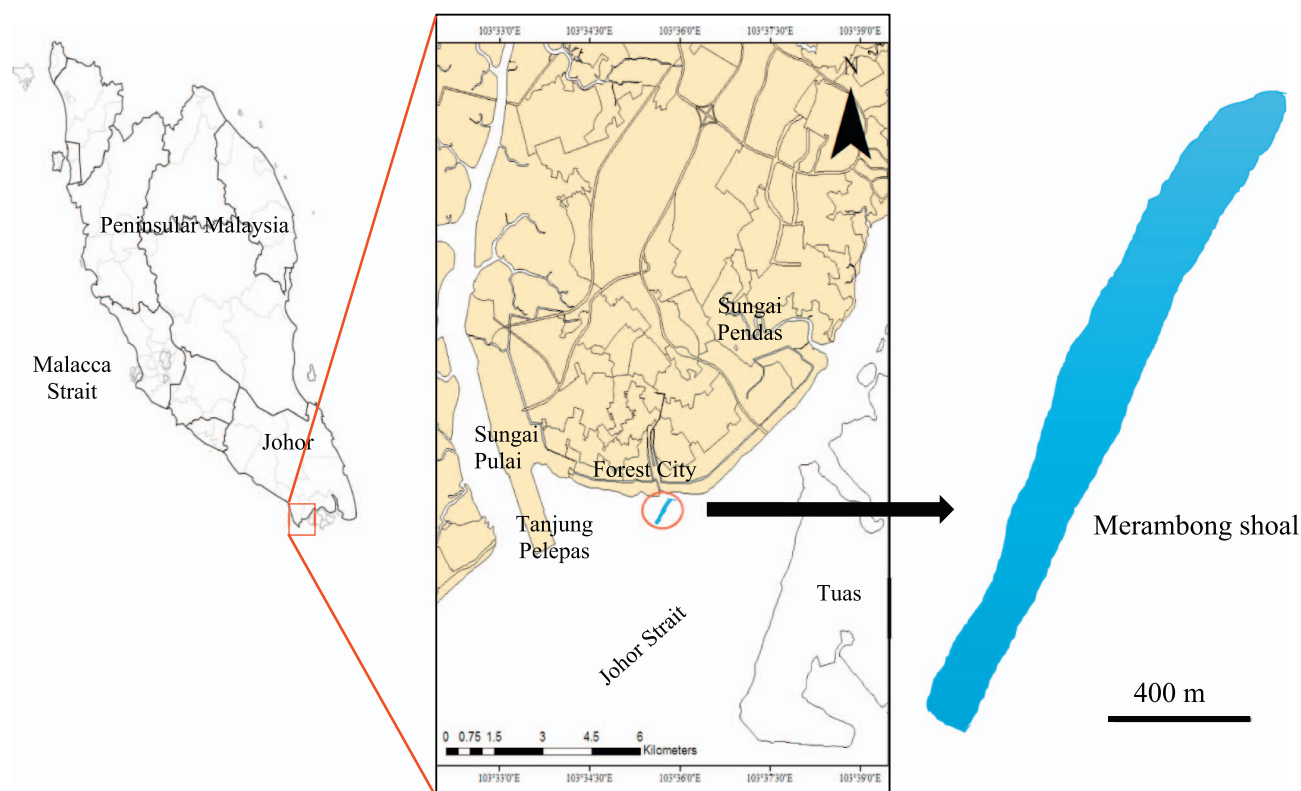


Fig. 1. Merambong shoal, Johor, Malaysia.

calcareous sandy mud subtidal shoal with depth of 2.0–2.7 m and only appears during low tides at 0–0.2 m (Japar Sidik and Muta Harah, 2003). Merambong shoal has the largest seagrass bed in Malaysia, with a length of 1–1.2 km and width of 100–200 m. It supports the highest number of seagrass species, with *E. acoroides*, *Halophila ovalis*, and *Halophila spinulosa* among the dominant species (Bujang et al., 2006). Merambong shoal is situated adjacent to the Malaysia–Singapore Second Link Bridge, Port of Tanjung Pelepas, and Tanjung Bin Power Plant. The shoal is about 5 km away from heavy industrial activities and incinerators in Tuas, Singapore. This shoal receives water from the Malacca Strait, Johor Strait, and the nearby stream, Sungai Pulas.

E. acoroides samples were collected from 20 sampling points in June 2014, when the shoal was exposed during the receding tide (< 2 m). The collected *E. acoroides* was rinsed with seawater to eliminate sediment that was attached to the rhizome and root. The samples were placed into individual acid-washed storage bags. Then, the collected samples were kept in a cool box (< 4 °C) during transit to the laboratory and preserved at – 20 °C prior to laboratory analysis.

In the laboratory, the *E. acoroides* were thawed, washed again with seawater collected from the sampling sites, and subsequently separated into root, rhizome, and blade. Epiphytes were removed from the blades by using a cleaned glass slide. The separated parts were oven dried at 60 °C until constant weight was achieved. The separated root, rhizome, and blade samples were ground separately in an agate mortar to obtain a homogenous powder. About 0.2 g of powdered part each was weighed and placed in a glass vial. Then, 5.0 mL of concentrated nitric acid (EMSURE® (65%), Merk Millipore, MA, USA) was added to the vial. The samples were digested in a single reaction chamber microwave acid digestion system (Ultrawave, Milestone, Sorisole, Italy), as described by Bertoldi et al. (2014). Firstly, the system was pre-pressurized with 40 bar nitrogen. The temperature was ramped up to 120 °C over 20 min and then increased to 250 °C over another 20 min. The total running time of the system was 40 min, with the maximum operating temperature and pressure of 250 °C and 160 bar, respectively. Then, the

chamber was depressurized after cooling down to 65 °C. Subsequently, the digested samples were filtered through a 0.45 µm cellulose acetate filter into a 50 mL polyethylene volumetric flask, which was then filled up with 2% HNO₃. The concentrations of dissolved metals (Cd, Cu, Ni, Pb, and Zn) in the filtrate were analysed via quadrupole inductively coupled plasma-mass spectrometry (ICP-MS; ELAN DRC-e, Perkin Elmer, Shelton). The sample blank and standard reference material (SRM) were prepared along with the samples in triplicate. The performance of the ICP-MS equipment was checked daily to ensure the instrument was operated in accordance with the manufacturer's specifications. Daily performance analysis of sensitivity (Mg, In, and U), interferences (oxides of Ce and doubly charged Ba), background, and precision were evaluated by using the SmartTune Solution for Standard ELAN/DRC-e. The ICP-MS operating conditions and measurement parameters are summarized in Table 1. Isobaric interferences were

Table 1
Instrumental parameters for trace metal determination using ICP-MS.

Instrument settings	Preferences
Instrument	ICP-MS, ELAN DRC-e, Perkin Elmer
ICP RF power, W	1250
Vacuum pressure, Torr	5.30×10^{-6}
Quadrupole rod offset Std (QRO)	0.5
Gas	Ar
Plasma gas flow rate, L min ⁻¹	17.00
Auxiliary gas flow rate, L min ⁻¹	1.20
Nebulizer gas flow rate (NEB), L min ⁻¹	0.98
Lens Voltage, V	7.10
Scanning mode	Peak hopping
Resolution	Standard
Dwell time, ms	50
Sweeps per reading	10
Number of readings per replicate	3
Sample was delay, s	30
Autosampler	ASX-520

Table 2

Isotopes of studied elements, the potential interferences and correction equations used to minimize isobaric and polyatomic interferences.

Element	Isotope	Correction equation	Potential interference
Cadmium	111	$^{114}\text{Cd} - (0.027250 \times ^{118}\text{Sn})$	$^{95}\text{Mo}^{16}\text{O}^+$
Copper	63	$^{63}\text{Cu} - (0.06 \times ^{47}\text{Ti})$	$^{31}\text{P}^{16}\text{O}_2^+, ^{47}\text{Ti}^{16}\text{O}^+$
Lead	208	$(1.000)(^{206}\text{Pb}) + (1.000)(^{207}\text{Pb}) + (1.000)(^{208}\text{Pb})$	$^{192}\text{Pt}^{16}\text{O}^+$
Nickel	60	$^{60}\text{Ni} - (0.003 \times ^{43}\text{Ca})$	$^{44}\text{Ca}^{16}\text{O}^+, ^{23}\text{Na}^{37}\text{Cl}^+$
Zinc	66	$-0.035313 \times ^{60}\text{Ni}$	$^{50}\text{Ti}^{16}\text{O}^+, ^{32}\text{S}^{17}\text{O}_2, ^{33}\text{S}^{16}\text{O}_2^{16}\text{H}^+$

avoided or minimized by eliminating the sample matrices, selecting suitable isotopes, and applying appropriate interference correction equations (Table 2).

Ultrapure grade chemicals were used in the sample preparation. Ultrapure water (water resistivity > 18.2 MΩ cm at 25 °C; Milli-Q, Millipore, MA, USA) was used for laboratory operations including reagent, blank, and standard calibration solution preparation. All glassware and apparatus were cleaned before use by soaking in 5% HNO₃ for 24 h and rinsing with Milli-Q water to ensure minimum mineral contamination. A series of standard calibration solutions with multiple concentrations were freshly prepared by diluting a stock standard calibration solution (Multi-element Calibration Standard 3, Perkin Elmer) with 2% HNO₃. The calibration curve for all elements showed good linearity over the concentration range, with correlation coefficients > 0.9999 (Table 3). Quality control was performed to inspect the ruggedness of the instrument by regularly checking with an external standard, which was the highest dilution prepared (lowest concentration) of the stock standard calibration solution, once every 10 samples analysed. The instrument was recalibrated and cleaned by using 5% HNO₃ whenever signs of drift were observed. The limit of detection (LOD) was calculated as three times the standard deviation of the signal of the sample blank. The limit of quantification (LOQ) was calculated as 10 times the standard deviation of the sample blank (Sanagi et al., 2009) (Table 3). The repeatability of the measurement method was illustrated by calculating the relative standard deviation (RSD) of the trace metal (Cd, Cu, Ni, Pb, and Zn) concentrations in the root, rhizome, and blade of *E. acoroides*. The % RSD of trace metal concentration measurement in the root, rhizome, and blade samples were 0.20–2.40%, 0.09–2.40%, and 0.20–4.60%, respectively. The standard reference material SRM 1547 (peach leaves) from the National Institute of Standards and Technology (NIST, Gaithersburg, USA) was used to validate the accuracy of the analytical procedure; the recovery of the trace metals fell within satisfactory ranges (80.61 to 106.25%) (Table 4). Spiked samples were prepared in triplicate for the root, rhizome, and blade of *E. acoroides*. The recoveries were calculated to determine the effect of the matrix on analyte recovery. The recoveries of multi-elements in the spiked solution were in the ranges of 83.40–115.70% for root, 83.70–116.10% for rhizome, and 82.30–103.00% for blade (Table 5).

Statistical analyses were performed by using IBM SPSS Statistics

Table 3

The limit of detection (LOD) (μg L⁻¹), limit of quantification (LOQ) (μg L⁻¹), standard calibration concentration (μg L⁻¹) and correlation coefficient (r) for trace metal determination.

Element	LOD (μg L ⁻¹)	LOQ (μg L ⁻¹)	Standard calibration concentration (μg L ⁻¹)	Correlation coefficient (r)
Cadmium	0.00554	0.01848	0, 10, 20, 40, 50, 100	0.99998
Copper	0.00055	0.00184	0, 10, 20, 40, 50, 100	0.99998
Lead	0.00001	0.00005	0, 10, 20, 40, 50, 100	0.99997
Nickel	0.00196	0.00654	0, 10, 20, 40, 50, 100	0.99998
Zinc	0.00324	0.01079	0, 10, 20, 40, 50, 100	0.99999

Table 4

Average concentrations (μg g⁻¹) of trace metal in the standard reference materials NIST, SRM 1547 (peach leaves).

Element	Certified	Measured	Recovery (%)
Cd	0.026 ± 0.003 ^b	0.027 ± 0.001	103.85
Cu	3.700 ± 0.400 ^a	3.790 ± 0.050	102.43
Ni	0.690 ± 0.090 ^a	0.590 ± 0.010	85.51
Pb	0.800 ± 0.030 ^a	0.850 ± 0.020	106.25
Zn	17.900 ± 0.400 ^a	14.430 ± 0.400	80.61

^a Certified value.

^b Estimated value.

Version 21 (NY, USA) statistical software. One-way analysis of variance (ANOVA) was applied to analyse the differences in the mean trace metal concentrations between plant parts. When the mean metal concentrations of the plant parts had $p < 0.05$, multiple comparisons were compared by using Tukey's post-hoc test.

Translocation factor (TF) expresses the mobility of the trace metal from root to rhizome, root to blade, and rhizome to blade. Higher TF value indicates a greater translocation capability (Deng et al., 2004). TFs were calculated based on Eq. (1) (Bonanno and Di Martino, 2016).

Translocation factor (TF) Eq. (1):

[TM] rhizome/[TM] root

[TM] blade/[TM] root

[TM] blade/[TM] rhizome

where [TM] rhizome, [TM] root, and [TM] blade are the concentrations (in μg g⁻¹ dry wt) of the particular trace metal in the roots, rhizomes, and blades of *E. acoroides*.

One-way ANOVA showed that the trace metals in seagrass plant parts varied significantly among the sampling points ($p < 0.05$). The ranges of trace metal concentrations in plant parts varied from 0.05–0.81 μg g⁻¹ (Cd), 1.62–27.85 μg g⁻¹ (Cu), 1.89–9.35 μg g⁻¹ (Ni), 0.69–4.16 μg g⁻¹ (Pb), and 3.44–35.98 μg g⁻¹ (Zn). These findings revealed that the roots for this species typically had the lowest concentrations of Cd, Cu, Ni, and Zn, but not Pb. The general metal distribution of *E. acoroides* was: Zn > Cu > Ni > Pb > Cd (Fig. 2). Notably, the mean concentration of Zn in all plant parts: roots (6.03 ± 1.83 μg g⁻¹), rhizomes (20.92 ± 5.26 μg g⁻¹), and blades (14.03 ± 4.07 μg g⁻¹), were the highest among other studied metals. Zn was the major pollutant in Merambong shoal followed by Cu. The potential sources of Zn and Cu might be from antifouling paint and oil and fuel discharge from navigation and cargo activities (Matthiessen et al., 1999) along Johor Strait, which is renowned as the Asia Petroleum Hub. Elements, such as Cd and Ni, are widely used in heavy industries including steel and nickel alloy production, electroplating (Khan et al., 2014), and dry battery production (Väyrynen and Salminen, 2012). It is possible for these metals to become entrapped, as the Merambong shoal is located near the busy shipping lane close to Johor Strait, which includes the Port of Tanjung Pelepas and Tuas Industrial Park. Moreover, massive developments of industrial activities in Johor and Singapore have led to air pollution. The Johor-Singapore Causeway and the Johor-Singapore Second Link Bridge receive high traffic volumes of vehicles, and the incinerators in Singapore released a very fine fly ash (0.04–100 μm) that contains toxic trace metals such as Al, Pb, and Zn (Wu and Ting, 2006). Most of these major constituents are produced because of high temperature during the combustion process and volatilize to the atmosphere. These volatile constituents will finally deposit onto the sediment and become assimilated by the seagrass.

The rhizome/root and blade/root translocation ratios were above 1.0 for all trace metals (Cd, Cu, Ni, and Zn) except Pb (Fig. 3). The TF > 1 indicates that the plant accumulates a higher concentration of trace metals in the rhizomes than in the roots, or higher concentrations of trace metals are in the blades rather than in the roots. The blade/rhizome translocation ratio was also above 1.0 for Cd and Ni, but not

Table 5
Recovery of trace metal from spiked root, rhizome and blade of *Enhalus acoroides*.

Element	Concentration added ($\mu\text{g L}^{-1}$)	Root			Rhizome			Blade		
		Measured mass fractions		Recovery (%)	Measured mass fractions		Recovery (%)	Measured mass fractions		Recovery (%)
		Unspike samples ($\mu\text{g L}^{-1}$)	Spiked sample ($\mu\text{g L}^{-1}$)		Unspike samples ($\mu\text{g L}^{-1}$)	Spiked sample ($\mu\text{g L}^{-1}$)		Unspike samples ($\mu\text{g L}^{-1}$)	Spiked sample ($\mu\text{g L}^{-1}$)	
Cd	10	0.32	8.66	83.40	0.29	8.66	83.70	2.17	10.40	82.30
	20	0.32	17.24	84.60	0.29	17.26	84.85	2.17	19.62	87.25
Cu	10	5.47	14.93	94.60	17.49	26.30	88.10	18.43	27.87	94.40
	20	5.47	24.23	93.80	17.49	36.21	93.60	18.43	37.99	97.80
Ni	10	8.15	18.09	99.40	6.15	15.98	98.30	15.94	25.12	91.80
	20	8.15	27.39	96.20	6.15	26.58	102.15	15.94	36.54	103.00
Pb	10	10.56	19.86	93.00	3.25	13.11	98.60	4.25	13.57	93.20
	20	10.56	29.03	92.35	3.25	22.88	98.15	4.25	23.87	98.10
Zn	10	14.27	25.84	115.70	17.70	29.31	116.10	51.45	59.82	83.70
	20	14.27	31.27	85.00	17.70	40.50	114.00	51.45	68.01	82.80

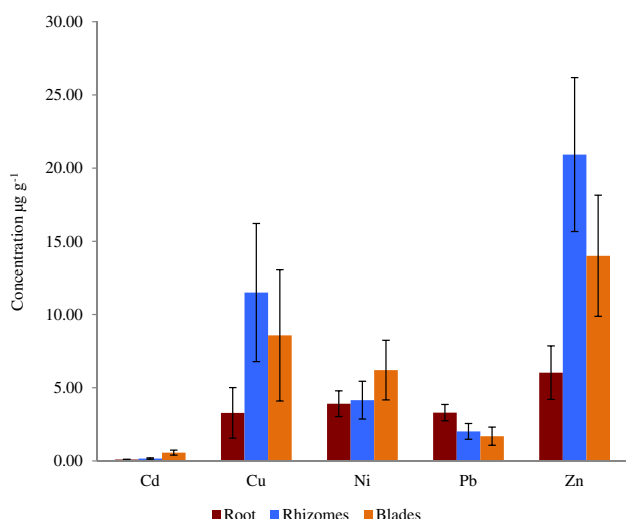


Fig. 2. Mean concentrations of trace metal in different plant parts of *Enhalus acoroides* from Merambong shoal.

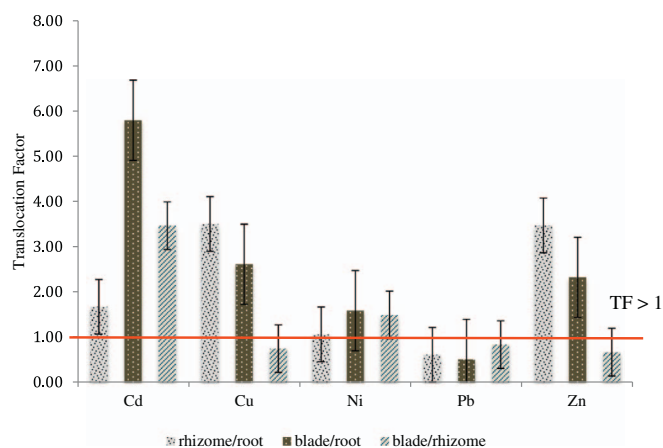


Fig. 3. Translocation factor (TF) of trace metal from root to rhizome, root to blade and rhizome to blade of *Enhalus acoroides*.

for Cu, Pb, and Zn (Fig. 3). The $TF < 1$ for Pb demonstrated that Pb translocation from roots to blades was limited. Pb is a non-essential element for plant growth. Therefore, seagrass might exclude Pb to reduce the metal's toxic impact. The plant might isolate this element in storage tissues, thus it limits mobilization of Pb to blades, where most

metabolic activity happens (Ambo-Rappe et al., 2011). The $TF > 1$ is a characteristic of hyperaccumulator plants, which can accumulate trace metals at a very high concentration in their above ground tissue (Tuapattinaya et al., 2016). Based on the calculated TF, *E. acoroides* is capable of translocating Cd, Cu, Ni, and Zn from roots to rhizomes and from roots to blades. In short, *E. acoroides* is a potential accumulator for Cd, Cu, Ni, and Zn.

In general, the range of trace metal concentrations in this study was comparable to those from other regions (Table 6). The concentrations of Pb in the plant parts of *E. acoroides* collected from the Pulau Estuary, Johor were reportedly $> 220.0 \mu\text{g g}^{-1}$ (Ahmad et al., 2014), which were higher than those found in this study and in any other regions. However, the concentration of Pb in the rhizome of *Cymodocea nodosa* from Western Mediterranean ($< 1.0 \mu\text{g g}^{-1}$) (Llagostera et al., 2011) and the blades of *Posidonia oceanica* from the Mediterranean ($1.4\text{--}1.8 \mu\text{g g}^{-1}$) (Lafabrie et al., 2007) were lower than the results obtained in the present study. The maximum concentrations of Cd and Ni in the present study were found to be similar with the maximum levels of those reported in other studies, except for the Pulau Estuary (Ahmad et al., 2015b) and Mediterranean (Lafabrie et al., 2007). The maximum concentrations of Cu observed in root, rhizome, and blade were in accordance with the maximum concentration of Cu in leaves of *Halophila ovalis* from Australia (Kilminster, 2013), in blades of *Cymodocea rotundata* from China (Li and Huang, 2012), and in blades of *E. acoroides* from Johor and China (Ahmad et al., 2015b; Li and Huang, 2012).

The present study shows the preliminary distribution of trace metals in the estuarine ecosystem of Merambong shoal, featuring the availability of these metals in the seagrass, *Enhalus acoroides*. This finding revealed that roots, rhizomes, and blades of *E. acoroides* are capable of accumulating trace metals (Cd, Cu, Ni, Pb, and Zn). Notably, rhizomes and blades accumulated the highest concentrations of Cd, Cu, Ni, and Zn. Translocation factors showed that this specific tape seagrass species is a hyperaccumulator plant, meaning it can accumulate trace metals (Cd, Cu, Ni, and Zn) at very high concentrations in its blades. Therefore, *E. acoroides* is a potential bioindicator of Cd, Cu, Ni, and Zn in estuarine ecosystems. However, it is not suitable as a bioindicator for Pb because *E. acoroides* limits the mobility of Pb to the entire plant in order to minimize the toxic impact of Pb on this species.

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Table 6

Ranges of trace metal concentrations ($\mu\text{g g}^{-1}$ dry weight) in various species of seagrass in comparison to those reported by previous studies.

Species	Plant part	Location	Pb	Cu	Zn	Ni	Cd	References
<i>Cymodocea nodosa</i>	Root	Ebri River Delta, Western Mediterranean	< 3.0	< 7.0	< 40.0	< 4.0	< 0.6	Llagostera et al. (2011)
	Rhizome		< 1.0	< 4.0	< 30.0	< 1.0	< 0.4	
	Leaf		< 4.0	< 8.0	< 60.0	< 4.0	< 4.0	
<i>Cymodocea rotundata</i>	Blade	Xincun Bay, China	10.0–15.5	10.6–19.1	32.7–54.6	–	1.5–2.9	Li and Huang (2012)
<i>Enhalus acoroides</i>	Whole plant	Pulau Estuary, Johor Strait	202.0–304.0	16.0–37.0	–	–	52.0–96.0	Ahmad et al. (2015b)
	Blade	Banten Bay, Indonesia	BDL	5.4–9.1	51.9–195.5	–	0.1–1.1	Suwandana et al. (2011)
	Blade	Xincun Bay, China	11.1–18.2	9.8–22.3	28.9–48.0	–	1.2–2.7	Li and Huang (2012)
	Blade	Pulau Estuary, Johor Strait	< 210.0	–	–	–	–	Ahmad et al. (2014)
	Blade	Merambong Shoal, Johor Strait	0.7–2.9	3.0–24.5	6.6–20.8	2.9–9.3	0.3–0.8	This study
	Rhizome	Pulau Estuary, Johor Strait	< 230.0	–	–	–	–	Ahmad et al. (2014)
	Rhizome	Merambong Shoal, Johor Strait	1.2–3.1	4.8–27.8	11.1–36.0	2.4–8.6	0.1–0.3	This study
	Rhizome & Root	Spermonde Islands, Indonesia	1.9–5.7	0.4–5.7	–	–	–	Werorilang et al. (2016)
	Root	Pulau Estuary, Johor Straits	< 180.0	–	–	–	–	Ahmad et al. (2014)
	Root	Merambong Shoal, Johor Straits	1.7–4.2	1.6–10.4	3.4–13.5	1.9–6.2	< 0.1	This study
<i>Halophila ovalis</i>	Leaf	Leschenault Estuary, Australia	–	< 23.5	< 30.2	< 3.0	< 0.3	Kilminster (2013)
	Rhizome	–	–	< 135.2	< 39.9	< 2.1	< 0.2	–
	Root	–	–	< 44.6	< 37.2	< 5.2	< 0.3	–
<i>Hadolue wrightii</i>	Whole plant	Todos os Sontos Bay, Brazil	–	2.2–13.4	13.1–39.5	2.0–9.9	0.1–1.7	Brito et al. (2016)
<i>Posidonia oceanica</i>	Blade	Canari, Mediterranean	1.4–1.8	–	–	27.5–60.3	2.1–5.4	Lafabrie et al. (2007)
<i>Ruppia megacarpa</i>	Leaf	Leschenault Estuary, Australia	–	< 59.2	< 29.7	< 1.7	< 0.3	Kilminster (2013)
	Rhizome & Root	–	–	< 45.8	< 33.5	< 4.1	< 0.2	–
<i>Thalassia hemprichii</i>	Blade	Xincun Bay, Hainan Island, China	9.8–12.6	9.0–12.8	49.7–63.6	–	2.6–3.2	Li and Huang (2012)
	Root	Ambon Island, Indonesia	–	–	–	–	0.9–1.0	Tuapattinaya et al. (2016)
	Leaf	–	–	–	–	–	0.2–1.2	–

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