UNIVERSITI PUTRA MALAYSIA

ASSESSMENT OF TRACE METALS IN SEAGRASS AREAS OF MERAMBONG SHOAL IN JOHOR, MALAYSIA

NORDIANI BINTI SIDI

FPAS 2020 19

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By

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Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfillment of the Requirements for the Degree of Master of Science

October 2019

Abstract of a thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Science

ASSESSMENT OF TRACE METALS IN SEAGRASS AREAS OF MERAMBONG SHOAL IN JOHOR, MALAYSIA

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October 2019

Chairman : Ahmad Zaharin Aris, PhD
Faculty : Environmental Studies

Seagrass bed and estuarine ecosystems are essential to the environment. Nevertheless, due to the massive development surrounding Johor Straits, it causes the estuary ecosystem at risk, which can be harmful to the health of the aquatic ecosystem. Meanwhile, most of the established studies of seagrass found in Malaysia were subjected to the biology and ecology field. Therefore, there was a knowledge gap in terms of trace metals profiling in the seagrass ecosystem. Thus, it is imperative to assess the trace metals pollution in the seagrass vegetated area of Merambong shoal, which focused on the distribution, enrichment and metals mobility of trace metals in surface sediment, pore water, and seagrass. In addition, this study aims to provide the baseline data on the physicochemical characteristics of surface sediment and pore water as well as the current status of pollution levels in these environmental matrices. Other than that, this study also assessed the potential of *Enhalus acoroides* as a bioindicator of trace metals. In order to achieve these objectives, 60 samples of surface sediment and pore water were randomly collected from 20 sampling points, which covered Merambong shoal area. The sampling campaign was conducted in June 2014 during the low tide. *E. acoroides* complete with roots, rhizomes, and blades were collected at the same location as the surface sediment and pore water were sampled. The collected surface sediment and pore water were measured for in-situ parameters (pH, salinity, TDS (Total Dissolved Solids) and electrical conductivity (EC)). The surface sediment and pore water also were analysed for chemical analyses (i.e., organic matter, cation exchange capacity, major anions and cations). Surface sediment, pore water, and *E. acoroides* were digested using acid digestion method for trace metals concentrations measurement. The concentrations of trace metals (i.e., cadmium (Cd), copper (Cu), lead (Pb), nickel (Ni), and zinc (Zn)) in collected samples were analysed by the inductive coupled plasma mass spectrometry (ICP-MS). In general, the physicochemical parameters (pH, salinity and EC) of surface sediment and pore water, the domination of sodium (Na^+) and chloride (Cl^-) in pore water and exchangeable of Na^+ in surface sediment were mainly influenced by the fresh-seawater mixing condition from nearby streams and Johor Straits. The influence of salinity from seawater also contributes to the classification of pore water as sodium chloride waters. The mean concentration of trace metals in surface sediment was in the following order: Zn>Pb>Cu>Ni>Cd while for pore water was as follows: Cu>Ni>Zn>Pb>Cd. The mean trace metals concentrations of *E. acoroides* was in the order of Zn>Cu>Ni>Pb>Cd. The irregular tidal wave and seawater current are the factors affecting the metals distribution. The highest concentration of Zn in surface sediment and seagrass is due to Zn is relatively abundant in the environment, essential micronutrients for plants and input from the anthropogenic activities. As for Soil-Water Partition Coefficient (K_d), Pb has high K_d value indicating higher retention and less mobility because inorganic lead compounds are commonly abundant in the sediment and do not dissolve in water under natural conditions. The concentration of Cd, Cu, Ni, Pb, and Zn in the surface sediment samples were very in low range and did not exceed the sediment quality guidelines values (i.e., Hong Kong Interim Sediment Quality Value (HK ISQV) low, Australian and New Zealand Environment and Conservation Council (ANZECC), Interim Sediment Quality Guidelines (ISQG) low, National Oceanic and Atmospheric Administration (NOAA) low and National Oceanic and Atmospheric Administration (NOAA) Effects Range Median (ERM)). Based on assessment via indices (i.e., Enrichment Factor (EF), Geoaccumulation Index (I_{geo}), and Contamination Factor (CF)), Pb showed significant enrichment in the surface sediment of Merambong shoal. The enrichment of Pb influenced by anthropogenic activities related to shipping and cargo activities. The value for The Risk Factor (Er^i) and Risk Index (RI) for surface sediment were <40 and <150. These ecological risk indices showed that the pollution and ecological risks of all trace metals were minimal. However, Interstitial Water Criterion Toxic Unit (IWCTU) revealed that all collected pore water samples could pose a risk of toxicity to overlying water, surface sediment, and aquatic organisms. The adverse environmental impacts that occur under sediment resuspension events lead to the toxicity of pore water in Merambong shoal. Bioconcentration Factor (BCF) revealed that *E. acoroides* have a significant ability to accumulate all studied trace metals from pore water. Each plant parts of *E. acoroides* showed different abilities in accumulating nutrients; blades showed the ability to accumulate more Cd and Ni while rhizomes for Zn and Cd, while roots for Pb. The biota-sediment accumulation factor (BSAF) suggested that *E. acoroides* were macroconcentrator of Cd, Cu, and Ni. Meanwhile, Translocation Factor (TF) revealed that *E. acoroides* is capable of translocating Cd, Cu, Ni, and Zn from roots to rhizomes and from roots to blades. *E. acoroides* control the translocation of Pb to the entire plant parts by isolate Pb in the roots to prevent growth inhibition and death. All of these suggested *E. acoroides* has potential as a bioindicator for Cd, Cu, Ni, and Zn, except for Pb. This study has successful provides a baseline of trace metals pollution status in the seagrass vegetated area. Through this study, the distribution and enrichment pattern of trace metals in surface sediment and toxicity of pore water also were further understood. This study also highlighted the potential of *E. acoroides* to be used as a bioindicator to reflect the trace metals pollution.

Keywords: seagrass, surface sediment, pore water, trace metals, bioindicator, bioconcentration factor (BCF), biota-sediment accumulation factor (BSAF), translocation factor (TF), Merambong shoal.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

PENILAIAN LOGAM SURIH DI KAWASAN RUMPUT LAUT BETING PASIR MERAMBONG DI JOHOR, MALAYSIA

Oleh

NORDIANI BINTI SIDI

Oktober 2019

Pengerusi : Ahmad Zaharin Aris, PhD
Fakulti : Pengajian Alam Sekitar

Ekosistem hamparan rumput laut dan muara adalah penting kepada alam sekitar. Walau bagaimanapun, disebabkan oleh pembangunan pesat sepanjang persekitaran Selat Johor, ia menyebabkan ekosistem muara berisiko yang boleh memudaratkan kesihatan ekosistem akuatik. Sementara itu, kebanyakan kajian rumput laut di Malaysia berkisarkan bidang biologi dan ekologi. Oleh yang demikian, terdapat jurang pengetahuan berkenaan profil pencemaran logam surih di dalam ekosistem rumput laut. Oleh itu, adalah penting untuk menjalankan penilaian mengenai pencemaran logam surih di kawasan hamparan rumput laut beting Merambong, yang memfokuskan kepada taburan, pengayaan dan pergerakan logam surih di dalam permukaan sedimen, air liang dan rumput laut. Tambahan pula, kajian ini bertujuan menyediakan data asas tentang ciri-ciri fiziko-kimia permukaan sedimen dan air liang serta status semasa berkenaan tahap pencemaran dalam matriks alam sekitar tersebut. Selain itu, kajian ini juga menilai potensi *Enhalus acoroides* sebagai bioindikator kepada logam surih. Untuk mencapai objektif ini, 60 sampel sedimen dan air liang diambil secara rawak daripada 20 lokasi pesampelan yang meliputi seluruh kawasan beting Merambong. Aktiviti persampelan telah dijalankan pada Jun 2014 semasa air surut. *E. acoroides* yang lengkap dengan akar, rizom dan daun diambil di lokasi yang sama dengan tempat persampelan permukaan sedimen dan air liang. Parameter in-situ (pH, saliniti, TDS (Jumlah Pepejal Terlarut) dan kekonduksian elektrik (EC)) bagi permukaan sedimen dan air liang diukur. Permukaan sedimen dan air liang turut dianalisa dengan ujian analisis kimia (iaitu, bahan organik, kadar pertukaran kation, anion dan kation). Permukaan sedimen, air liang dan *E. acoroides* dicerna menggunakan kaedah pencernaan asid bagi tujuan pengukuran kepekatan logam. Kepekatan logam surih (iaitu kadmium (Cd), kuprum (Cu), plumbum (Pb), nikel (Ni) dan zink (Zn) yang terdapat di dalam sampel tersebut dianalisa menggunakan instrumen spektrometri massa plasma bersama induktif (ICP-MS). Secara amnya, parameter fiziko-kimia (pH, saliniti dan EC) permukaan sedimen dan air liang, dominasi ion sodium (Na^+) dan klorida (Cl^-) air liang dan kation Na^+ permukaan sedimen adalah dipengaruhi oleh kesan pencampuran air sungai dan air laut dari sungai-sungai berdekatan dan Selat Johor. Saliniti juga turut mempengaruhi klasifikasi air liang sebagai ‘sodium chloride waters’. Purata kepekatan logam surih di dalam permukaan sedimen

adalah dalam turutan berikut, Zn> Pb> Cu> Ni> Cd manakala untuk air liang adalah seperti berikut: Cu> Ni> Zn> Pb> Cd. Purata kepekatan logam surih *E. acoroides* adalah dalam turutan Zn> Cu> Ni> Pb> Cd. Pasang surut air laut yang tidak menentu serta arus gelombang ombak merupakan faktor yang mempengaruhi taburan logam surih. Kepekatan Zn yang tinggi di dalam permukaan sedimen dan rumput laut disebabkan oleh kandungan Zn yang tinggi di dalam persekitaran secara semula jadi, merupakan nutrien penting bagi tumbuh-tumbuhan dan input daripada aktiviti antropogenik. Bagi Pekali Partisi Tanah-Air (K_d), Pb mempunyai nilai K_d yang tinggi di mana ia lebih cenderung untuk retensi dan minimakan pergerakan kerana sebatian bukan organik biasanya terdapat di dalam sedimen dan ia tidak larut dalam air dalam keadaan semula jadi. Kepekatan Cd, Cu, Ni, Pb dan Zn dalam sampel sedimen sangat rendah dan tidak melebihi nilai garis panduan kualiti sedimen (i.e., Hong Kong Interim Sediment Quality Value (HK ISQV) low, Australian and New Zealand Environment and Conservation Council (ANZECC), Interim Sediment Quality Guidelines (ISQG) low, National Oceanic and Atmospheric Administration (NOAA) low and National Oceanic and Atmospheric Administration (NOAA) Effects Range Median (ERM)). Berdasarkan penilaian menerusi indeks (i.e., Faktor Pengayaan (EF), Indeks Geo-akumulasi (I_{geo}), and Faktor Kontaminasi (CF)), Pb menunjukkan pengayaan yang ketara dalam sampel sedimen dari beting Merambong. Pengayaan Pb dipengaruhi oleh aktiviti antropogenik yang berkaitan dengan aktiviti perkapalan dan kargo. Nilai faktor Risiko (Er^i) dan Indeks Risiko (*RI*) untuk permukaan sedimen adalah <40 dan <150. Indeks risiko ekologi (Er^i and *RI*) menunjukkan bahawa pencemaran dan risiko ekologi untuk semua logam surih adalah minima. Walaubagaimanapun, Unit Toksik Kriteria Air Interstitial (IWCTU) mendedahkan semua sampel air liang boleh mendatangkan risiko toksiksiti terhadap air, sedimen dan organisma akuatik. Ketoksikan air liang di tebing Merambong berlaku disebabkan faktor kesan tindak balas pemendapan sedimen. Faktor biokonsentrasi (BCF) mendedahkan bahawa *E. acoroides* mempunyai keupayaan yang ketara untuk mengumpul logam surih dari air liang. Setiap bahagian tumbuhan *E. acoroides* menunjukkan keupayaan berlainan dalam akumulasi nutrien, daun menunjukkan keupayaan untuk mengumpul lebih banyak Cd dan Ni manakala rizom untuk Zn dan Cd manakala akar untuk Pb. Faktor akumulasi biota sedimen (BSAF) menunjukkan bahawa *E. acoroides* adalah makrokonsentrator terhadap Cd, Cu dan Ni. Sementara itu, Faktor translokasi (TF) mendedahkan bahawa *E. acoroides* mampu memindahkan Cd, Cu, Ni, dan Zn dari akar ke rizom dan dari akar ke daun. *E. acoroides* mengawal translokasi Pb ke seluruh bahagian dengan mengasingkan Pb di bahagian akar bagi menghalang perencatan pertumbuhan dan kematian. Berdasarkan penemuan ini, *E. acoroides* mempunyai potensi sebagai bioindikator untuk Cd, Cu, Ni, dan Zn. Kajian ini telah berjaya menyediakan data asas pencemaran logam surih bagi kawasan hamparan rumput laut. Melalui kajian ini, corak taburan dan pengayaan logam surih dalam sedimen serta ketoksikan air liang juga lebih difahami. Kajian ini juga menyerlahkan potensi *E. acoroides* untuk digunakan sebagai bioindikator terhadap pencemaran logam surih.

Kata kunci: rumput laut, sedimen, air liang, logam surih, bioindikator, faktor biokonsentrasi (BCF), faktor akumulasi biota-sedimen (BSAF), faktor translokasi, beting Merambong.

ACKNOWLEDGEMENTS

In the name of Allah, the most beneficent, the most eternally merciful. Alhamdulillah, thankful to the Almighty Allah S.W.T for granting me strength, patience, and courage in completing this research. I would like to express my most profound appreciation to my supervisor, Prof. Dr. Ahmad Zaharin Aris, who has the attitude and the substance of a genius, as he continually conveyed a spirit of adventure about research, and excitement about teaching. Without his guidance and persistence help, this dissertation would not have been possible.

I would like to thank my co-supervisor, Dr. Ferdius@Ferdaus Mohamat Yusuff who always shares the knowledge and gives constructive comments on my study. I extend my sincere appreciation to the lecturers from the Faculty of Environmental Studies (FPAS), particularly to Head Department of Environmental Sciences (from the year 2013 to 2019) and Associate Prof. Dr. Latifah Abdul Manaf, who always support and share their expertise. I would also like to acknowledge Universiti Putra Malaysia for financial assistance throughout my study.

Thank you to my dearest lab mates; Dr. Looi Ley Juen, Dr. Noorain, Ms. Nor Farhanna Mokhtar, Mrs. Nasyitah, Ms. Afifah, Ms. Hanisah, Ms. Wee Sze Yee, Dr. Tuan Mohd Fauzan, Dr. Shah Chistiani, Mr. Annuar and Mr. Razni who had lent their hand to the fullest.

Deepest appreciation to all the FPAS laboratory mates for their advice, idea, knowledge, practical and mental support during my lab works especially Mr. Tengku Shahrul Tengku Md. Yusoff, Mr. Shamsuddin Johan, Mr. Zamani Ismail, Mr. Zairi Ismail, Miss Norlela Talib, Mrs. Siti Arina Mahat and Mrs. Yuhanira Yunus. My appreciation also dedicated to administration staff of Pejabat Timbalan Dekan (Pembangunan dan Siswazah), Pejabat Am Jabatan Sains Alam Sekitar and Pejabat Am Dekan, who always assisting me during the completion of this Master's research.

My special appreciation is extended to my beloved parents, Sidi Anang and Noorma Awang for their endless support and blessing. Lastly, the warmest thank you to my other half, Muhammad Idham Said, and my kids, Kholiq, Zahra and Nadine, for the sacrifices, encouragement and inspiration.

Finally, my greatest thanks to FPAS postgraduates and colleagues in Universiti Putra Malaysia, who always support and assisting me during my study. I wish to extend my sincere appreciation and best wishes to all of you. Thank you to all that have directly or indirectly contributed to the completion of this study.

This thesis was submitted to the Senate of the Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

Ahmad Zaharin Aris, PhD
Professor
Faculty of Environmental Studies
Universiti Putra Malaysia
(Chairman)

Ferdius@Ferdaus Mohamat Yusuff, PhD
Senior Lecturer
Faculty of Environmental Studies
Universiti Putra Malaysia
(Member)

____
ZALILAH MOHD SHARIFF, PhD
Professor and Dean
School of Graduate Studies
Universiti Putra Malaysia

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Signature: ______________________________
Name of Chairman of Supervisory Committee: Professor Dr. Ahmad Zaharin Aris

Signature: ______________________________
Name of Member of Supervisory Committee: Dr. Ferdius@Ferdaus Mohamat Yusuff

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LIST OF ABBREVIATIONS

ANZECC	Australian and New Zealand Environment and Conservation Council
ASTDR	Agency for Toxic Substances and Disease Registry
APHA	American Public Health Association
BCF	Bioconcentration Factor
BSAF	Biota-Sediment Accumulation Factor
Ca	Calcium
Cd	Cadmium
CEC	Cation Exchange Capacity
CF	Contamination Factor
Cu	Copper
EC	Electrical conductivity
Ef	Enrichment Factor
Eh	Redox Potential
EPA	Environmental Protection Agency
ERL	Effect Range Low
ERM	Effect Range Medium
FAAS	Flame Atomic Absorption Spectrophotometer
FCVs	Final chronic value
ICP-MS	Inductive Coupled Plasma Mass Spectrometry
I_{geo}	Geo-accumulation index
IWCTU	Interstitial Water Criterion Toxic Unit
LOI	Loss on Ignition
ISPQ	Interim Sediment Quality Guidelines
ISQV	Interim Sediment Quality Value
K	Kalium or Potassium
LOD	Limit of Detection
LOQ	Limit of Quantification
Mg	Magnesium
Na	Natrium or sodium
NI	Nemeraw Index
Ni	Nickel
NIST	National Institute of Standards and Technology
NTU	Nephelometric Turbidity Unit
Pb	Plumbum or Lead
SPSS	Statistical Package for Social Science
SRM	Standard Reference Material
TDS	Total Dissolve Solid
TF	Translocation Factor
USEPA	United State Environmental Protection Agency
Zn	Zinc

LIST OF UNITS

%	percent
<	less than
≤	less or equal to
>	more than
≥	more or equal to
°C	degree celsius
$mS\ cm^{-1}$	milliSiemens per centimeter
µm	micrometer
mv	millivolt
g	gram
$mg\ kg^{-1}$	milligram per kilogram
$\mu g\ kg^{-1}$	microgram per kilogram
$mg\ L^{-1}$	milligram per liter
$\mu g\ L^{-1}$	microgram per liter
km^2	square kilometer
m	meter
mm	millimeter
cm	centimeter
km	kilometer
ppt	part per trillion
meq	milliequivalents
meq/100g	milliequivalents per 100 grams
et al.	And other

CHAPTER 1

INTRODUCTION

1.1 Background of the Study

About 3.7 billion inhabitants live in the coastal area all over the world (Rêgo et al., 2018). To date, about 40% of the world's population resides within 100 km of the coast (United Nations, 2017). By 2050, it is predicted that the human population will increase to more than 9 billion will live in a coastal area (United Nations, 2017). In Malaysia, Kuala Terengganu, Penang, Kota Kinabalu, and Johor Bahru are part of developed cities that settled along the coastal strip. Throughout history, cities were constructed around ports, and people take advantage of the amenities, including food supply, jobs, transportation, defensible position, and trades opportunity that the ports offered. Apart from that, most people attracted to live in the coastal area as it provides tourism attractions that draw nature and water sports lovers, retirees, or urban residents looking for a vacation home (El Barmelgy and Rasheed, 2016). Therefore, constructions in the coastal area continue to develop to satisfy people's demand.

The rapid urbanisation of the coastal region leads to an increase in anthropogenic activities such as deforestation, dredging and soil reclamation, aquaculture, and industrial activities. These urbanisation-related activities are threatening the productive areas of the coastal and marine ecosystem, including mangrove forests, coral reefs, and seagrass beds. These ecosystems received the resulting industry run-off, atmospheric deposition, and municipal sewage discharges from the cities and agriculture activities. As a result, the ecosystem would be negatively affected by various types of pollutants, including nutrients and trace metals.

Trace metals pollution has raised concern from researchers all over the world for decades because of its persistency since they cannot be degraded or destroyed (Asati et al., 2016). Prolonged exposure to trace metals could be carcinogenic and toxic that harmful to living organisms' health and further pollute the environment, thus affecting the food chain (Jaishankar et al., 2014). The enrichment of trace metals in surface sediment of the estuarine and coastal ecosystem is subjected as the primary concern. The elevation of trace metals enrichment in estuarine and coastal sediment occurs each year because sediment is an excellent sink for pollutants (Goher et al., 2014).

Several studies found that accumulations of trace metals tend to occur in estuary areas that are situated close to sources of trace metals pollutions, such as industrial zones (Liu et al., 2016; Chen et al., 2016; Silva et al., 2017; Mehr et al., 2017). Nonetheless, the distribution of trace metals in aquatic environments generally, influenced from natural processes (e.g., weathering process, soil, coastal and seafloor erosion and biological activities) and various anthropogenic activities (e.g., effluents, discharge of wastewater from cities and industrial zones) (Khatri and Tyagi, 2015). Sediment also acts as a nonpoint source of pollution and has the potential to release the sediment-bound metals and other pollutants to overlying waters and later adversely affect aquatic organisms (Wang et al., 2010).

The distribution of trace metals pollution in aquatic environments can be estimated by analysing water, sediments, and aquatic organisms (El Nemr et al., 2012). Numerous studies have been conducted to evaluate the degree of trace metals contamination in aquatic environments such as sediment (Zulkifli et al., 2010; Haris and Aris, 2015) and aquatic water (Looi et al., 2013). However, these approaches were designed to gauge the environmental quality of marine ecosystems but did not provide information on the bioavailability of the elemental fractions nor ecotoxicology aims and require periodic analyses (Fränzle, 2006). Aquatic organisms such as fish, mussel, snail, and algae are commonly being utilised as bioindicators of trace metals contamination (Sobihah et al., 2018; Dabwan and Taufiq, 2016; Haris, 2015; Chakraborty et al., 2014). The contamination in tissues of living organisms contributes knowledge in terms of time integrated measurement of trace metals bioavailability. Besides, it also could give early predictions of any environmental disturbance (Rainbow, 1995).

In marine biodiversity, the seagrasses ecosystem is part of productive ecosystems that have a vital role in the coastal and marine environment worldwide. Seagrasses are crucial to marine life due to the ability to produce oxygen through photosynthesis. These marine angiosperms store carbon (Githaiga et al., 2017), improve water quality (York et al., 2017), absorb dissolved metals (Macinnis-Ng and Ralph, 2002) and very good at giving an indicator of the surrounding pollution (Thangaradjou et al., 2010). They are critical habitat for marine life and profit maker for the fishing industry. The estimated annual economic value of seagrass in 2009 was $3,500 per hectare (Loney, 2009). However, the function of seagrass in the marine ecosystem is often neglected. The number of seagrass species is declining each year due to estuarine and coastal development. The losses of seagrass beds are the size of a soccer field every thirty minutes, globally (UMCES, 2009).

1.2 Problem Statement

Johor Straits is located between Johor and Singapore provincial hubs that connect the east and the western world geographically, making this strait as the busiest shipping routes both eastbound and westbound (Rizzo and Glasson, 2012). In the vicinity of the Johor Straits, there is a shipping port, Tanjung Pelepas Port, which is ranked in the top 20 major container ports in the world (Subhan and Ghani, 2008). Johor Straits is rapidly developing, with the opening of the Iskandar Corridor area, land clearing and land reclamation projects took place in the waters of the Straits of Johor.

In 2014, Forest City development began rising in the Johor Straits off the southwestern coast of Johor (Rahman, 2017). This city portrayed as a role model for future cities that had initially been designated as an utterly industrial area, centered around Port of Tanjung Pelepas, the Tanjung Bin Power Plant, and the Tanjung Bin International Maritime Centre (Rahman, 2017). Along the Johor Straits, there are many activities involving heavy industries and aquaculture activities. The surrounding mangroves were cut down to expand further the Tanjung Bin coal fire power plant.

Merambong shoal is situated approximately 1.0 km southern of Tanjung Kupang, adjacent to the Pulai River estuary and mangrove forest of Tanjung Piai (Misbari and Hashim, 2015). This shoal receives water sources from a nearby river. The river flow to the water level is feared to flow contaminated water from industrial waste and sewage discharge. Merambong shoal has the largest seagrass bed in the country that provides a dynamic ecosystem of marine treasures, including seagrass, dugongs, bivalves, fish, seaweed, and seahorse (Bujang et al., 2006).

Seagrass bed in Sungai Pulai estuary and Tanjung Adang Darat was once at risk in 1998 and almost disappeared in 2003 (Bujang et al., 2006). It was a consequence of land reclamation activity for the development of new port facilities and dredging of the shallow shipping passageways. On the other hand, the siltation and pollution caused by coastal development in the area, which includes Forest City, Port of Tanjung Pelepas, and the Tanjung Bin power plant, had given a negative impact on seagrass inhibits this shoal (Ahmad, 2016). As a result, there was a significant loss of seagrass species such as *Halophila minor*, *Cymodocea rotundata,* and *Syringodium isoetifolium,* which home to threatened animals such as dugongs, turtles, and seahorses (Muta Harah et al., 2014).

In recent years, scientific research on seagrass as a potential bioindicator in trace metals pollution has been conducted in Vietnam (Nguyen et al., 2017), Italy (Bonanno and Di Martino, 2016), Brazil (Brito et al., 2016), Indonesia (Tupan and Azrianingsih, 2016), China (Li and Huang, 2012), and Australia (Kilminster, 2013) due to its capacity to integrate biological physical and chemical parameters (Orlando-Bonaca et al., 2015). These studies focus mostly on trace metals concentrations in the seagrass compartment and relate with their capability as a bioindicator. In Malaysia, a study on seagrass is mostly subjected to biology and the ecological field. Therefore, there is limited research on the accumulation of trace metals in seagrass and also the metals pollution level of the seagrass ecosystem.

Apart from that, it is hypothesized that pore water is the primary source of nutrients for seagrass growth (Fourqurean et al., 1992). This study will improve the knowledge about the role of *E. acoroides* roots, rhizomes and blades, in which the nutrient uptake mechanism from pore water and sediment can be updated. This study also provides a comprehensive understanding of the trace metals mobility in a particular environment. Considering that is a lack of baseline data on trace metals content in seagrass and also its surrounding environment, an extensive baseline study on trace metals pollution especially in the seagrass ecosystem, is urgently needed.

The NABC (Needs, Approach, Benefits, and Challenges) analysis was conducted in the present study to identify the knowledge gaps of the previous research that needed to be addressed regarding the monitoring studies of trace metals in the Merambong shoal (Table 1.1). Besides, the NABC analysis could also assist the present study to overcome the possible limitations of the previous studies. The analysis showed there are needs to conduct continuous monitoring as the Merambong shoal is continuously exposed to the sea-based and land-based pollution. Therefore, trace metals pollution levels and the health status of the study area can be updated frequently. In order to achieve these objectives, several approaches will be implemented. The approaches involved were the analytical chemistry experiments, the application of analytical instruments, exploration of multiple indices and calculations. Other than fulfilling the knowledge gap on several issues related to trace metals pollution, particularly in surface sediment, pore water, and seagrass of Merambong shoal, this study could help authorities' researchers and interest parties for future monitoring planning and developing regulations. The proper standard operating procedure and several precautions should be taken into account to overcome all the challenges during the sampling campaign and sample analysis.

Table 1.1: The outputs of needs, approaches, benefits, and challenges (NABC) analysis for recent studies on trace metals concentrations and distribution in the Merambong shoal.

	Outputs
Needs	• Continuous monitoring studies are needed as the Merambong shoal is continuously exposed to sea-based and land-based pollution. • Baseline data on physicochemical properties of sediment and pore water • Baseline data on trace metals contamination in the seagrass environment (surface sediment, pore water, and seagrass) is needed. • Information on the metals mobility via the plant and environment matrices (sediment and pore water) • Information on the trace metals pollution status of surface sediment and pore water is needed to assess the degree of trace metals contamination. • Application of potential bioindicators to reflect the health status of the estuarine and coastal environment.
Approaches	• Determination of physicochemical characteristics of sediment and pore water. • Determination of the concentration of selected trace metals in surface sediment, pore water, and seagrass (roots, rhizomes, and blades). • Determination of metals mobility using calculations (Soil-Water Partition Coefficient, K_d and Translocation Factor, TF) • Determination of trace metals pollution status of surface sediment and pore water via applying various indices (sediment quality guidelines (SQG), Enrichment Factor, Contamination Factor, Geoaccumulation Index, Ecological Risk Assessment and interstitial water criterion toxic units (IWCTU)). • Determination of the *E. acoroides* as a potential bioindicator for trace metals monitoring study based on several factors such as bioconcentration factor (BCF) and biota-sediment accumulation factor (BSAF)

Benefits	• Provide information on the physicochemical characteristics of environment matrices (sediment and pore water) in the seagrass vegetated area. • Address the lack of information on trace metals concentrations and distribution in the Merambong shoal environment. • Provide information on current trace metals pollution status in Merambong shoal. • Provide better insights into the current degrees of trace metals pollution of Merambong shoal, thus will help authorities' researchers and interest parties for future planning.
Challenges	• Samples, especially pore water, might be challenging to analyse due to the physicochemical characteristics easily change. Analyses should be conducted immediately after the sampling campaign.

1.3 Research Questions

The following research questions are listed to achieve the research objectives:

1. What are the physicochemical parameters of surface sediment and pore water, and their relationship between the parameters and trace metals concentrations?
2. What are the concentrations of trace metals in surface sediment, pore water, and parts of *E. acoroides* (roots, rhizomes, and blades) and their relationship in respective study sites?
3. What is the trend of trace metals mobility between surface sediment and pore water and, in the plant parts itself?
4. What is the pollution level in surface sediment and pore water of Merambong shoal?
5. What are the bioconcentration factor and biota-sediment accumulation factor of trace metals in roots, rhizomes, and blades of *E. acoroides*?

1.4 Research Objectives

In general, this study aimed to determine the concentration and the distribution of trace metals in the seagrass vegetated area of Merambong shoal, Johor, Malaysia. The specific research objectives are as follows:

1. To determine the physicochemical parameters of surface sediment and pore water of Merambong shoal, Johor.
2. To determine the concentration of trace metals in the surface sediment, pore water, and parts of *E. acoroides* of Merambong shoal, Johor.
3. To correlate the relationship of the physicochemical parameters between the parameters and trace metals concentrations of the sample matrices (surface sediment, pore water and, parts of *E. acoroides*.

4. To explore the mobility of trace metals between compartments (surface sediment and pore water) and within plant parts (roots, rhizomes, and blades) using Soil-Water Partition Coefficient (K_d) and Translocation Factor (TF).
5. To assess the pollution level in surface sediment and pore water using several indices (e.g., Enrichment Factor (EF), Contamination Factor (CF), Geoacccumulation Index (I_{geo}), Ecological Risk Assessment (Er^i and *RI*) and Interstitial Water Criterion Toxic Unit (IWCTU)).
6. To ascertain the bioconcentration factor (BCF) and biota-sediment accumulation factor (BSAF) of trace metals in parts of *E. acoroides* (roots, rhizomes, and blades).

1.5 Scope of the Study

This study covers the following scopes:

1. This study focused on the concentration and distribution of trace metals such as Cd, Cu, Pb, Ni, and Zn in the surface sediment, pore water, and parts of *E. acoroides* (roots, rhizomes, and blades) of Merambong shoal, Johor. The physicochemical parameters of surface sediment and pore water were measured to achieve a better understanding of the dynamic interactions that influence the metals distribution in the estuarine area, especially the seagrass bed environment.
2. This study explored the mobility of trace metals in the sample matrices (surface sediment and pore water) and within plant parts (roots, rhizomes, and blades). This input will give a better understanding of metals behaviour in the studied samples.
3. This study applied various indices (e.g., Enrichment Factor, Contamination Factor, Geoaccumulation Index, Ecological Risk Assessment, Interstitial Water Criterion Toxic Unit) were measured to elucidate the enrichment, pollution, risk, and toxicity of metals concentration in the surface sediment and pore water. The application of these indices assists in determining the threat level to biota and the ecosystem. The value will give a better understanding of evaluating the exposure of hazardous pollutants to seagrass.
4. This study employed statistical analyses (e.g., Pearson correlation analysis, Cluster analysis), which help in the determination of the relationship between trace metals concentrations in the surface sediment, pore water, and parts of *E. acoroides.*
5. This study also ascertains the ability of *E. acoroides* to accumulate trace metals from surface sediment and pore water by measuring bioconcentration factor (BCF) and biota-sediment accumulation factor (BSAF). These measurements would aid in the determination of *E. acoroides* as a potential bioindicator.

1.6 Significance of the Study

The data obtained in this study reflect the contamination level of trace metals in the seagrass ecosystem area of Merambong shoal (e.g., surface sediment, pore water, and seagrass). This study could generate the information on the capability of *E. acoroides* parts (roots, rhizomes, and blades) in accumulating trace metals, translocating trace metals to its entire parts, and the ability of seagrass to reflect the contamination from its surroundings. This study improves the knowledge about the role of *E. acoroides* roots, rhizomes, and blades, in which the nutrient uptake mechanism from pore water and surface sediment can be updated. This study also provides a comprehensive understanding of the trace metals mobility in a particular environment. In addition, this study could contribute to developing guidelines related to heavy metals monitoring programs in the future by utilizing the seagrass species.

1.7 Research Framework of the Study

The research framework developed for this study is depicted in Table 1.2.

Table 1.2: The research framework for recent studies on trace metals concentrations and distribution in the Merambong shoal.

Issues and justification	Research questions
• Lack of background information on physicochemical characteristics of environment matrices (sediment and pore water) in the seagrass vegetated area • Inadequate data on trace metals content in seagrass species and environment matrices (surface sediment and pore water) from seagrass vegetated area • The correlation between the physicochemical parameters and the concentration of Cd, Cu, Ni, Pb, and Zn of the studied samples from Merambong shoal is not well studied. • Insufficient information on metals mobility within the seagrass and environment matrices of seagrass vegetated area (sediment and pore water) • Lack of data on pollution level status in seagrass vegetated area • Feasibility of *E. acoroides* act as a bioindicator	• What are the physicochemical parameters of surface sediment and pore water and their relationship between the parameters and trace metals concentrations? • What are the concentrations of trace metals in surface sediment, pore water, and parts of *E. acoroides* (roots, rhizomes, and blades) and their relationship in respective study sites? • What is the trend of trace metals mobility between surface sediment and pore water and, in the plant parts itself? • What is the pollution level in surface sediment and pore water of Merambong shoal? • What are the bioconcentration factor and biota-sediment accumulation factor of trace metals in roots, rhizomes, and blades of *E. acoroides*?

Objectives	Activities	Findings
• To determine the physicochemical parameters of surface sediment and pore water of Merambong shoal, Johor.	• Collection of surface sediment and pore water samples from 20 sampling points • Measurement of the physicochemical parameter of surface sediment and pore water using in-situ equipment	• Discover the physicochemical characteristics of studied matrices
• To determine the concentration of trace metals in the surface sediment, pore water, and parts of *E. acoroides* of Merambong shoal, Johor.	• Collection of surface sediment and pore water samples from 20 sampling points • Analysis of trace metals content in surface sediment, pore water and seagrass using ICP-MS	• Discover the concentration and distribution pattern of trace metals in respective samples
• To correlate the relationship of the physicochemical parameters between the parameters and trace metals concentrations of the sample matrices (surface sediment, pore water and, parts of *E. acoroides*	• Exploration of the physicochemical and trace metals data from Merambong shoal respective samples	• Discover the relationship between the parameters and trace metals concentrations of sample matrices (surface sediment, pore water, and seagrass)
• To explore the mobility of trace metals between compartments (surface sediment and pore water) and within plant parts (roots, rhizomes, and blades)	• Application of indexes such as Soil-Water Partition Coefficient, K_d • Application of Translocation Factor (TF) calculation	• Discover the distribution coefficient, K_d for Soil-Water Partition Coefficient • Discover the capability of parts of *E. acoroides* to translocate the metals
• To assess the pollution level in surface sediment and pore water	• Application of indexes such as Enrichment Factor (EF), Contamination Factor (CF), Geoacccumulation Index (Igeo), Ecological Risk Assessment (Er^i) and	• Defined the pollution status of surface sediment and pore water • Defined the ecological risk status at the respective study area

	RI), and Interstitial Water Criterion Toxic Unit (IWCTU).	
• To ascertain the bioconcentration factor and biota-sediment accumulation factor of trace metals in parts of *E. acoroides* (roots, rhizomes, and blades)	• Application of various calculation (BCF and BSAF)	• Revealed the capability of *E. acoroides* as a bioindicator.

1.8 Thesis Outline

This thesis is divided into five (5) chapters that consist of an Introduction (Chapter 1), Literature Review (Chapter 2), Materials and Method (Chapter 3), Results and Discussion (4), and Conclusion and Recommendations (5). The description of each chapter are as follows:

Chapter 1 provides a brief introduction to the urbanisation and development in the estuarine and coastal area, the importance of seagrass in terms of ecological function, and the role of seagrass. The problem statements, the objectives, the scope of the study, and the significance of the study also covered in this chapter. The analysis of Needs, Approaches, Benefits, and Challenges (NABC) was performed to encompass the needs of this study

Chapter 2 elaborates on the past literature reviews regarding several relevant topics and also prior research that almost similar to this study.

Chapter 3 discussed the detailed procedure of the materials and methods used during sampling, sample preparation, sample digestion, trace metals analysis, and data analysis. Calculations on various indices to assess the pollution level on surface sediment and pore water also explained.

Chapter 4 covers the results and discussion for findings from analysed samples. Some parts of the findings in this chapter were published in journal and conference proceedings (see the list of publications at the end of this thesis). In general, this chapter provides an overview of the current status of surface sediment, pore water and *E. acoroides* in regards to the physicochemical properties and metals concentration. Besides, the pollution level posed by metals in surface sediment and pore water and several indices (i.e., Bioaccumulation Factor (BCF), Biota-sediment Accumulation Factor (BSAF) and Translocation Factor (TF)) applied in this study were also discussed. Other than that, the relationships between metals in surface sediment and pore water with metals in plant parts of *E. acoroides* and the capability of this species act as a bioindicator were further explored.

Chapter 5 summarized the conclusion of the findings made in this study based on the objectives of Chapter 1. Recommendations for future research on this topic were also proposed. The analysis of Strength, Weakness, Opportunity and Threat (SWOT) was performed to overcome the limitations for a comprehensive and holistic monitoring approach of trace metals pollution in the future.

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