SEAGRASS INTERACTION WITH HEAVY METALS AT PULAI RIVER ESTUARY

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requirements for the award of the degree of
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This thesis is dedicated to my family.

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ABSTRACT

Environmentalists have raised their concerns that pollution from development along Pulai River Estuary will have an impact on marine ecosystem. In 1994 eleven seagrass species were found in the area. However, when this study were conducted in 2011 only seven seagrass species were identified at the area, namely *Enhalus acoroides, Halophila minor, Halophila spinulosa, Halophila ovalis, Thalassia hemprichii*, *Halodule uninervis* and *Cymodocea serrulata.* The seagrass can uptake metals and therefore plays the role as bioindicator. Field work was conducted between July 2011 and April 2014 where seagrass, water and sediment were collected for analysis. The samples were analysed using Perkin Elmer Atomic Absorption Spectrophotometer Model AAnalyst 400 for copper (Cu), cadmium (Cd), and lead (Pb). Flow Injection Mercury System Perkin Elmer model FIMS 100 was used for mercury (Hg) and arsenic (As) analysis. Analysis of variance and Pearson's correlation coefficients of metal concentrations were carried out using Statistical Package for the Social Science (SPSS) for seagrass tissues, seawater and sediment. Esri ArcGIS software was used to determine the metals distribution. The seagrass percent covers on the seagrass bed were determined by transect method. The study shows that *Halophila minor* was the most abundant species covering Pulai seagrass bed at 27% followed by *Halophila ovalis* (18%), *Halophila spinulosa* (8.8%), *Enhalus acoroides* (6.4%), *Thallasia hemprichii* (5.3%), *Cymodocea serrulata* (1%), and *Halophila uninervis* (0.3%). Among the seven seagrass species found, *Halophila ovalis* have the highest accumulation of metal and indicates positive significant correlation to translocation of metal in seagrass tissues, hence it meets the criteria to be selected as a bioindicator. Mapping using Esri ArcGIS, shows the metals distribution originated from land use. Monitoring conducted on 4^{th} of April, 2014 indicated that land reclamation for Forest City has changed the condition of seagrass bed hydrodynamic and trophic state from upper-mesotrophic to light-eutrophic. Quantitative water, sediment and seagrass fugacity/equivalance mass balanced model was developed to describe the movement pattern of metals that ends up in the seagrass bed. Estimation rates of As, Cu, Cd, Hg and Pb concentration in seawater are at 3.18 µg/L, 32.35 µg/L, 39.94 µg/L, 4.99 µg/L and 99.86 µg/L, respectively for 1 day.

ABSTRAK

Pencinta alam sekitar sering melahirkan kebimbangan mereka tentang pencemaran disebabkan oleh pembangunan di sepanjang muara Sungai Pulai yang akan memberi kesan kepada ekosistem marin. Pada tahun 1994, sebelas spesies rumput laut dikenal pasti di kawasan tersebut. Walau bagaimanapun, apabila kajian ini dilakukan pada 2011 hanya tujuh spesies rumput laut dikenal pasti di kawasan tersebut iaitu *Enhalus acoroides, Halophila minor, Halophila spinulosa, Halophila ovalis, Thalassia hemprichii, Halodule uninervis* dan *Cymodocea serrulata.* Rumput laut boleh mengambil logam dan oleh itu memainkan peranan sebagai petunjuk biologi. Kerja lapangan telah dijalankan antara Julai 2011 dan April 2014 di mana sample rumput laut, air dan sediment telah diambil untuk analisis. Sampel tersebut telah dianalisis menggunakan Spektrofotometer Serapan Atom model Pekin Elmer AAnalyst 400 untuk kuprum (Cu), kadmium (Cd) dan plumbum (Pb). Sistem suntikan aliran mercuri model Perkin Elmer FIMS 100 telah digunakan untuk analisis mercuri (Hg) dan arsenik (As). Analisis varians dan pekali korelasi Pearson bagi kepekatan logam telah ditentukan menggunakan Pakej Statistik untuk Sains Sosial (SPSS) untuk tisu rumput laut, air laut dan sedimen. Pemetaan menggunakan Perisian Esri ArcGIS telah digunakan bagi penentuan taburan logam. Peratus rumput laut di atas beting pasir rumput laut ditentukan menggunakan kaedah transek. Kajian menunjukan bahawa *Halophila minor* merupakan spesies dengan litupan terbanyak di atas benting pasir Sungai Pulai pada 27% diikuti oleh *Halophila ovalis* (18%), *Halophila spinulosa* (8.8%), *Enhalus acoroides* (6.4%), *Thalassia hemprichii* (5.3%), *Cymodocea serrulata* (1%), dan *Halophila uninervis* (0.3%). Antara tujuh spesies rumput laut yang dijumpai, *Halophila ovalis* mempunyai penumpukan logam paling tinggi dan menunjukkan korelasi yang signifikan dan positif kepada translokasi logam dalam tisu rumput laut, oleh itu ia memenuhi kriteria untuk dipilih sebagai petunjuk biologi. Pemetaan menggunakan Esri ArcGIS, menunjukkan taburan logam adalah berasal daripada guna tanah. Pemantauan yang dijalankan pada 4 April 2014 menunjukkan bahawa penambakan tanah untuk Forest City telah mengubah keadaan hidrodinamik dan trofik benting pasir daripada mesotrofik-tinggi kepada eutrofik-rendah. Model kuantitatif fugasiti/keseimbangan jisim telah dibangunkan bagi air, sedimen, dan rumput laut untuk menggambarkan corak taburan logam yang berakhir di atas beting pasir. Anggaran kadar bagi kepekatan As, Cu, Cd, Hg dan Pb dalam air laut adalah 3.18 μg/L, 32.35 μg/L, 39.94 μg/L, 4.99 μg/L dan 99.86 μg/L untuk satu hari.

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LIST OF ABBREVIATIONS

SJER	-	South Johor Economic Region
AAS 400	-	Atomic Absorption Spectrophotometer Model AAnalyst 400
ANOVA	-	Analyses of variance
SPSS	-	Statistical Package for the Social Science
SG	-	Seagrass bed
CR	-	Reef
MG	-	Mangrove
PEL	-	Pelagic
IUCN	-	International Union for Conservation of Nature
SI	-	Surface irradiance
GIS	-	Geographic Information System
MMWQS	-	Malaysia Marine Water Quality Standard
ISQGs	-	Interim Marine Sediment Quality Guidelines
PELs	-	Probable Effect Levels
GPS	-	Global Positioning System
DO	-	Dissolved oxygen
ASEAN	-	Association of Southeast Asian Nations
PVC	-	Poly vinyl chloride
Ea	-	*Enhalus acoroides*
Hm	-	*Halophila minor*
Hs	-	*Halophila spinulosa*
Ho	-	*Halophila ovalis*
Th	-	*Thalassia hemprichii*
Hu	-	*Halodule uninervis*
Cs	-	*Cymodocea serrulata*
Wp	-	Whole plant
Rh	-	Rhizomes

Rt	-	Root
Sh	-	Shoot
Ulva sp.	-	Ulva specie
n	-	Number of sample
r	-	Correlation coefficient
p-value	-	Significance level
SE	-	Standard error
P	-	Input
Q	-	Output
A_{ij}	-	Flow of the chemical from compartment i to j
M_i	-	Mass of Compartment i
C_i	-	Concentration of the chemical in compartment i
d/dt	-	First derivative
$C_i^{t+\Delta t}$	-	Concentration of compartment i at time t+Δt
C_i^t	-	Concentration of compartment i at time t
Δt	-	Discretization of time

LIST OF SYMBOLS

ºC	-	Celsius
µm	-	Microgram
g	-	Gram
mL	-	Millilitre
mm	-	Millimetre
cm	-	Centimetre
m	-	Meter
km	-	Kilometre
ha	-	Hectare
ppm	-	Parts per million
mg/L	-	Milligrams per litre water
µg/L	-	Microgram per litre
µg/g DW	-	Microgram per gram Dry Weight
N	-	Northern
E	-	Eastern
°	-	Degrees
'	-	Minutes
"	-	Seconds
%	-	Percent
AS	-	Arsenic
Cu	-	Copper
Cd	-	Cadmium
Hg	-	Mercury
Pb	-	Lead
O_2	-	Oxygen

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CHAPTER 1

INTRODUCTION

1.1 Introduction

Seagrasses are rooted angiosperms marine plants widely distributed in large areas known as seagrass beds (Kirkman, 1990; McKenzie *et al*., 2001; Vermaat *et al*., 2004; Bastyan and Cambridge, 2008). Seagrass beds are the most productive plant communities, they play ecological role as 'nursery habitats' in coastal ecosystems that supply food and shelter to vulnerable marine organisms such as sea cucumbers, starfish, seahorses, and thereby maintain a diverse biodiversity (Duarte and Chiscano, 1999; Waycott *et al*., 2005; Jackson *et al*., 2006; Lee *et al*., 2007; Eklöf *et al*., 2008; Choo *et al*., 2009).

Researchers from around the world are continuously searching for new seagrass species. Since the past decade, many issues have been raised on seagrasses, while documentation of seagrasses are still ongoing, some species have already been reported lost due to dredging activities (Erftemeijer and Robin Lewis III, 2006). It was reported that one way to measure the degree of pollution in estuaries is by using seagrasses as bioindicator for metal contamination (Schlacher-Hoenlinger and Schlacher, 1998; Prange and Dennison, 2000; Ferrat *et al*., 2003). Researchers also concur that seagrasses can be used rapidly to reflect the overall health of coastal waters due to their sharp sensitivity and variation in the environment (Nienhuis, 1986; Warnau *et al*., 1995; Costantini *et al*., 1991; Bortone and Turpin, 2000; Ferrat *et al*., 2003; Macinnis-Ng and Ralph, 2004). Seagrasses have remarkable metal bioaccumulation capacity because they interact directly with water column and pore water through leaves and roots as ionic uptake (Vermaat *et al*., 2004; Macinnis-Ng and Ralph, 2004; Llagostera *et al*., 2011). Furthermore, seagrass leaves cleans seawater by absorbing dissolved metals while their roots play a role in shoreline protection by reducing coastal erosion from raging storms (Macinnis-Ng and Ralph, 2002). Tropical seagrasses with the greatest diversity were identified in the Indo-Pacific region (Short *et al*., 2001; Short *et al*., 2011). However, the continuously increasing pollution will kill-off the seagrasses 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).

'The Convention on Wetlands of International Importance' (commonly known as the Ramsar Convention, 1971) is generally understood to be the pioneer global agreement on nature conservation (Matthews, 1993). Ramsar established the first globally coordinated institutional framework for conservation of a threatened ecosystem and set the standard for major global conservation treaties that followed, such as the Convention on Migratory Species in 1983 and the Convention on Biological Diversity in 1993 (Hettiarachchi *et al*., 2014). The Ramsar Convention is a core part of the international biodiversity governance system. Ramsar Site is a protected area with international importance and need to avoid an anthropogenic impact (Bellio and Kingsford, 2013). Pulai River which is the study area chosen for this study was listed as Ramsar site on 31^{st} January 2003.

An estuary is known as the most valuable aquatic ecosystem with certain areas having seagrass beds that house extensive marine biodiversity (Danovaro and Pusceddu, 2007; Selleslagh *et al*., 2012; Liquete *et al*., 2013). Aside from biodiversity, economic growth can be generated in the vicinity of estuaries i.e. the development of ports, petrochemical hubs, cities and residential areas (Rizzo and Glasson, 2012). Due to increasing demand for coastal resources as well as human population growth, the coastal ecosystem is exposed to a wider variety of pollutants. They are exposed to anthropogenic contaminants including complex mixtures of heavy metals from industrial, agricultural and domestic waste, arriving via rivers or through atmospheric deposition (Lafabrie *et al*., 2007; Gillet *et al*., 2008; Villate *et al*., 2013; Emelogu *et al*., 2013). In addition, human activities such as coastal land disturbance, motor boating and dredging contribute to pollution (Burkholder *et al*., 2007; Lewis *et al*., 2007). Furthermore increasing rate of enrichment of organic matter in an ecosystem causes eutrophication (Nixon, 1995).

The public and environmentalists have often raised their concerns on the excessiveness of nutrients and heavy metal pollution around estuaries. Seagrasses can be also act as bioindicator to reflect the overall health of coastal waters because of their sharp sensitivity to variations in the environment. On top of that, they can also remove dissolved metal from seawater and sediment. In fact, this has raised curiosity and interest of many researchers. However, little supporting evidence on the ability of tropical seagrasses has been recorded. As stated by Ooi *et al*., (2011), data recorded for metal accumulation in tropical seagrasses in Southeast Asia is very limited. Therefore, this study was conducted to further explore the effectiveness of seagrasses in absorbing dissolved metals from their surroundings.

1.2 Background of Study

1.2.1 Case Study Locations

Two areas were selected in this study which is the upstream of Pulai River and seagrass bed of Pulai River Estuary. Figure 1.1 shows the aerial map of study location. Pulai River has several tributaries with associated mangrove, intertidal mudflats and inland freshwater that represent lowland tropical river basin. Pulai River flows from Mount Pulai up to the Johor Straits in which the seagrass bed of Pulai River Estuary is located. The seagrass bed of Pulai River Estuary is the largest in Malaysia with an approximate area of 3.15 km^2 (Nisha, 2008). Besides Johor Straits, Malacca Straits and Singapore Straits also contributes to water that flows into the seagrass bed at Pulai River Estuary.

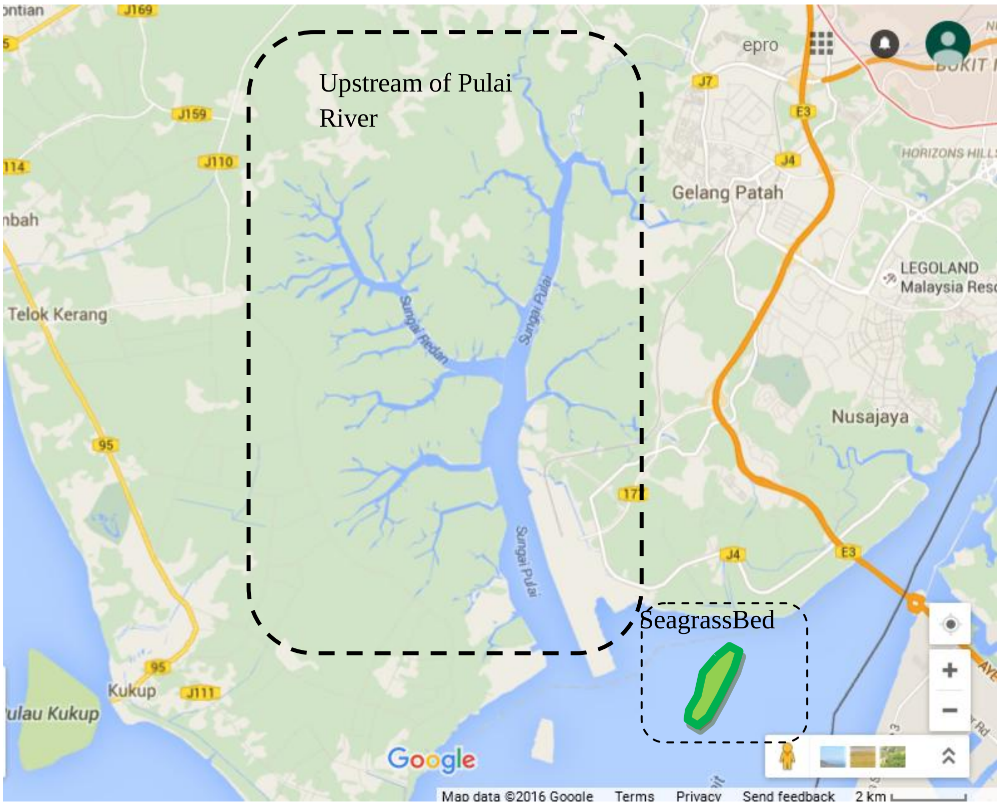


Figure 1.1 Location of study area

Seagrass bed of Pulai River Estuary is located between two powerful regional hubs of Johor and Singapore. Hence, its location is geographically strategic on the world's busiest shipping routes eastbound and westbound (Rizzo and Glasson, 2012). Furthermore, it is also surrounded by many projects such as Port of Tanjung Pelepas, Tanjung Bin Power Plant, Asia Petroleum Hub and development area from Tuas, Singapore as shown in Figure 1.2. The distance between Port of Tanjung Pelepas to the seagrass bed is 4 kilometers. The distance between Asia Petroleum Hub to the seagrass bed is 5.5 kilometres. The distance between Tuas, Singapore to the seagrass bed is 3 kilometers. Thus the seagrass bed is surrounded with pollution area.

1.3 Statement of Problem

Currently, there are still ongoing construction at the Pulai River Estuary due to expansion of Port of Tanjung Pelepas, urbanizations and industrialisation. Port activities causes enhancement of shipping transits eastbound and westbound. Figure 1.2 shows the picture of shipping transits at mouth of Pulai River. Johor Port Authority plans to expand the port-handling operations and related activities at the Port of Tanjung Pelepas in 2016, hence Port of Tanjung Pelepas currently handled about 10.5 million tonnes of cargo yearly and the volume would rise to 15 million tonnes yearly when the project completed in 2019 (The Star, 2015). The increasing cargos have the probability to cause accidents between container cargos.

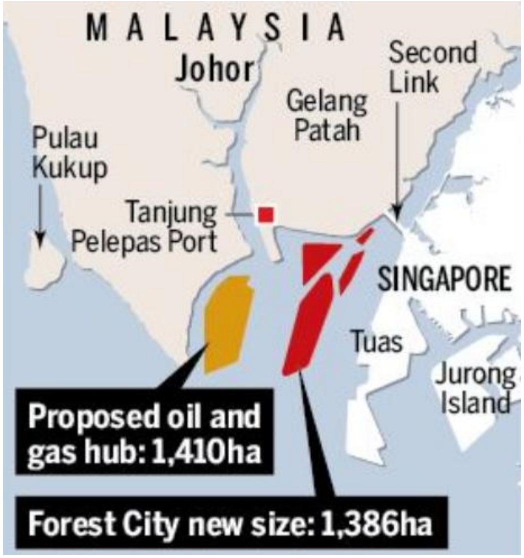
It was reported that within 10 years period from 2000 to 2010, 31% out of overall transit involved accidents of container cargo around Johor Straits (Rusli, 2012). Casual container cargo breakdowns are due to collision, foundering, fire or exploration (George, 2008). Hence cargo accidents contribute to major oil, hazardous and noxious substance spill incidents entail adverse impacts on the marine environment. The accidents need to be clean up urgently however clean up operation process is costly and depends on capacity of oil spills.

Figure 1.2 Shipping transits at Pulai River Estuary

Currently development at Iskandar Malaysia is swift with various undergoing projects. The latest development is Forest City, a mega-project with an artificial island of nearly 2000 hectares or half the size of Putrajaya which is located in the Johor Straits (Maznah, 2014). The huge island was reported to be a collaboration between renowned China developer Country Garden Holdings (based in Guangdong) and Johor state-owned People Johor Infrastructure Group (Nigel, 2014). The master plan was approved by Iskandar Malaysia, yet this project was not listed in the proposed South Johor Economic Region (SJER) master plan as in Flagship Zone C: Western Gate Development (Mahdzir, 2014).

In 2015, construction and reclamation for Forest City to be built on a man-made island in the Johor Strait was approved by Malaysia Department of Environment as long as they ensure that all compliance monitoring in terms of air, noise, water quality and sediment are robustly implemented and carried out. In order to comply with Malaysia Department of Environment standards, the total size of Forest City was slightly reduced from 1,623 hectares to 1,386 hectares and divided into four reclaimed islands instead of one huge island (Marissa Lee, 2015). The proposed revised size of Forest City and the new size of Forest City is shown in Figure 1.3. Even though Forest City is divided into four reclaimed island, the development will still effect seawater hydrodynamic. Apart from that, nearby the seagrass bed is also the expansion of 1,410 hectares reclamation project for an oil and gas hub. Thus, these construction activities are causing increase amounts of pollution via coastal land disturbance, dredging and accessibility improvement work.

a)



b)

Figure 1.3 Reclamation area of Forest City that overlaps with Pulai River Estuary seagrass bed a) proposed reclamation area of Forest City (Azlan 2014), b) new Forest City with four islands after approval from Malaysia Department of Environment (Marissa Lee, 2015)

Seagrass bed of Pulai River Estuary helps to provide shelter and breeding grounds for various vulnerable marine animals. Figure 1.4 shows type of marine animals that inhabit the seagrass bed. The example of marine animals are spotted seahorse, knobbly sea star, smooth sea cucumber, alligator pipefish, white-spotted rabbit fish, spotted scat, blue-spotted fantail ray, fan-bellied filefish, sea urchin and etc. In fact there are vulnerable species facing high risk of extinction at seagrass bed such as coastal horseshoes crab, tiger-tail seahorse, and spotted seahorse (Choo *et al*., 2009). Environmentalists and public including Singaporean have raised concerns that pollution occurring at the development site along Pulai River Estuary will affect the aesthetics and ecosystem especially in sensitive areas like the seagrass bed. These marine animals should be protected to ensure that they are not extinct for the sake of future generations.

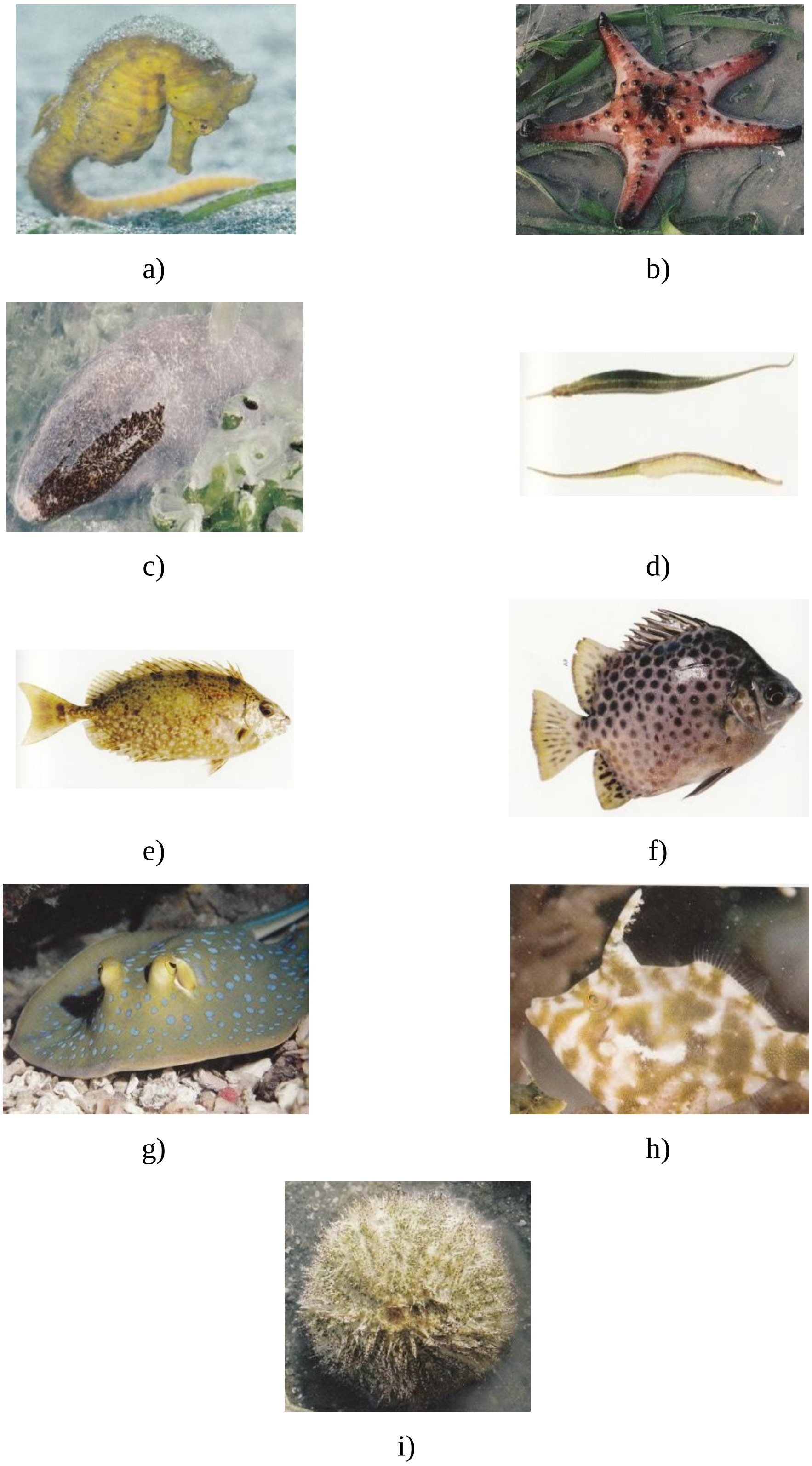


Figure 1.4 Example of marine animals found at seagrass bed, a) spotted seahorse, b) knobbly sea star, c) smooth sea cucumber, d) alligator pipefish, e) white-spotted rabbit fish, f) spotted scat, g) blue-spotted fantail ray, h) fan-bellied filefish, i) sea urchin (Choo *et al*., 2009)

Pollution disrupts marine life's lifecycle. As stated by Bahadori and Vuthaluru (2010), pollution is associated with low dissolved oxygen concentrations in water, which disturbs marine ecosystem and cause fish mortality, odours and anaerobic conditions. In fact, the population of vulnerable animals for examples seahorse and pipefish at seagrasss bed Pulai River Estuary were reported to decrease from 2007 to 2009 (Choo, 2009). In 2007, the number of seahorses is 170 unfortunately in 2009 the number of seahorses decreased to 46. On the other hand, the number of pipefish also decreased from 2008 to 2009 which is only 41. Table 1.1 shows the data recorded on the number of seahorses and pipefish during low tide. The data was counted during low tide condition because it is easier to catch seahorses and pipefish during low tide compared to high tide condition.

Table 1.1 : Summary of seahorses and pipefish in 2007, 2008, and 2009 during low tide (Choo, 2009)

Species	Seahorse			Pipefish		
Year	2007	2008	2009	2007	2008	2009
Male	92	77	19	53	63	20
Female	77	69	26	27	44	19
Juvenile	1	-	1	1	2	2
Total	170	146	46	81	109	41

In addition, increase in pollution around Pulai River Estuary can also decrease seafood supply. As we know, fish are the most abundant in the seagrass bed, hence fisherman rely on surrounding seagrass bed to earn a living (Unsworth *et al*., 2010). The detail number of fish on type of fish found is attached in Appendix A. Data was requested from Jabatan Perikanan Johor Bahru. There are several types of fish caught by fisherman such as black pomfret, siver pomfret, mullet, shad, marine catfish, jewfish, mixed fishes, cahcunda shad, grouper, dorab wolf-herring, ray, catfish eel, barramundi and barred Spanish mackerel. The main catches of fish at Johor Straits are jewfish, marine catfish and mullet. Figure 1.5 shows data of fish catch from 2009 to 2014. The number of fish in unit metric ton from year 2009 to 2014 is 1321, 1324, 1275, 1269, 1133 and 890, yearly. From the bar chart, it was found that the numbers of fish at Johor Bahru district continuously decreased from year 2010 until 2014. It is believed that the decreasing numbers of fish is due to progressive reclamation projects at Johor Straits. Hence this prove that reclamation project contributes to pollution in seawater and can disturbs marine ecosystem.

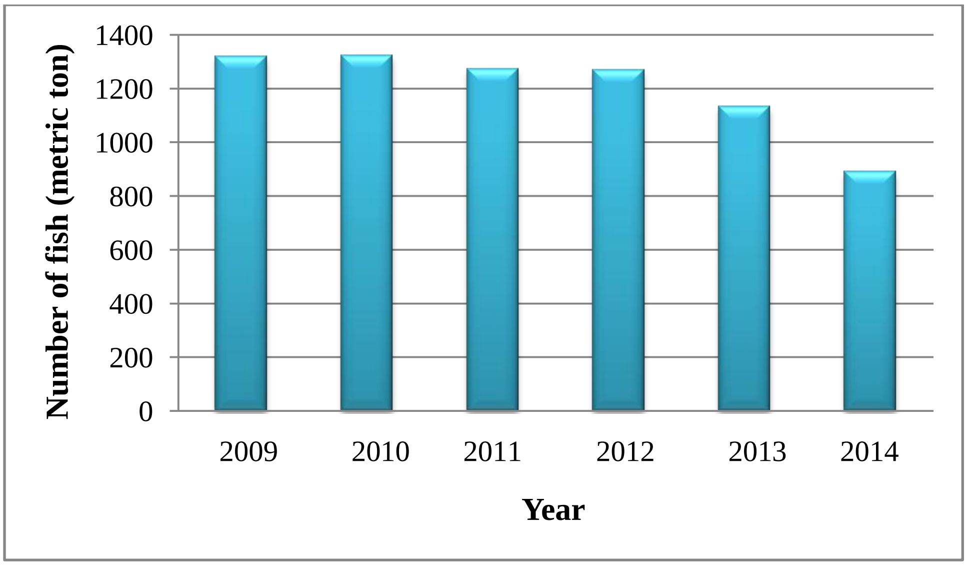


Figure 1.5 Fish landings in Johor Bahru area from 2009 to 2014

1.4 Objectives of Study

Objectives of the study are achieved:

i. To identify seagrass species and it's coverage on the seagrass bed.

ii. To determine the amount of accumulated metals in different part of seagrass species, sediment and seawater. The metals investigated are arsenic (As), copper (Cu), cadmium (Cd), mercury (Hg) and lead (Pb).

iii. To determine the correlation of metal content between seagrass tissues, sediment and seawater, in order to ascertain seagrass as metal indicator.

iv. To identify landuse activities in the study area and movement pattern of metals that ends up in the seagrass bed.

1.5 Scope of Study

In-situ parameters which include dissolved oxygen, salinity, temperature, pH, chlorophyll-a and secchi depth were measured and results were compared with the guidelines of National Marine Water Quality Standard for Malaysia. In this study, seagrasses was observed and identified at the seagrass bed. The percentage cover of each species on the seagrass bed was estimated using transect method (0.5 m x 0.5 m) with data recorded into a datasheet. Three types of samples (seagrasses, sediment and seawater) were collected from the seagrass bed. The samples were collected, digested and analyzed using Atomic Absorption Spectrophotometer (AAS) for accumulation of As, Cu, Cd, Hg and Pb.

Data of seagrasses, sediment and seawater obtained were further analysed to identify variances and correlations. Analysis of variance (ANOVA) and Pearson's correlation coefficients for seagrasses, sediment and seawater were carried out using Statistical Package of Social Science (SPSS) version 16. In addition, the correlations of metal concentration between different parts (leaves, rhizomes and roots) of the seagrass have also been analysed in order to attain the metal translocation pattern in the entire plant system. This is one of the factors of consideration in selecting the most suitable bioindicator. Seawater samples were collected from 17 different locations in the catchment area of Pulai River Estuary. The samples were taken near to the sources of pollution. The metal content of the seawater samples were also analysed using AAS. Data were then exported to ArcGIS software for data interpolation by kriging method with confidence interval from the area of land use around Pulai River Estuary. Thus, the interaction fugacity model for water, sediment and seagrass be obtained.

There are also some limitations in this study; i.e, seagrass samples could only be collected during low tide; sampling could only be carried out during day time and therefore monthly analysis could not be achieved due to irregular tide level. Sampling had to be stopped immediately whenever the weather turned bad due to safety reasons as conditions like strong wind and heavy rain or thunderstorm that is unsafe for field works.

1.6 Significance of Study

This study has produced insight knowledge on selection of the most suitable seagrass species as a bioindicator. Seagrass are sensitive to changes in seawater quality, and therefore can be a valuable indicators of metals and reflect the overall health of an estuarine ecosystem. Moreover observation for the decrease intensity of seagrasses in coastal waters is an easy and practical way to monitor pollution. Less intensity of seagrass cover indicates high intensity of pollution. In fact, it can be monitored by educating the local community (fisherman and villagers) to be watchful on pollution. However, equipment used to measure the intensity of pollution in seawater and sediment are excessively expensive and requires a certain level of proficiency to operate. Hence the concept of observation on monitoring seagrass ability to indicate pollution would be an easy task.

1.7 Organization of Thesis

This thesis consists of six chapters, Chapter 1 is the introduction part. It describes the background of this study related to the benefits of seagrasses and the pollution caused by development projects. Chapter 2 consist of literature review, which discuss the issues and information gathered by various researchers. It also gives an overview of the current state of seagrass including guidelines on standards for seawater, sediment and seagrass. In Chapter 3, methodology describes appropriate research designs, procedures, instrumentations, data processing and software used to analyze the samples. Chapter 4 discuss the results obtained from the field work and experiments conducted. This chapter summarize the collected data and discuss major findings: identification of seagrass species, length and width of seagrass bed and percentage of seagrass distribution. It also describes the amount of metals and their correlations in seawater, sediment and seagrasses. The selections of seagrass species as bioindicator was also determined.

Chapter 5 describes the water quality due to land use, the impact of land use and land reclamation on Pulai River Eatuary. The seagrasses might perish and vulnerable marine animals can be extinct due to excessive project construction work in the area. Seawater, sediment and seagrass interaction fugacity model was also developed at Pulai River Estuary. Finally, conclusions are presented in Chapter 6 together with recommendations for future research.

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