Water Quality Assessment of Downstream Penang River and Heavy Metals Accumulation in Seagrass

by

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Dissertation submitted in partial fulfilment of
the requirements for the
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CERTIFICATION OF APPROVAL

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Approved by,

_____

(Dr. Lavania Baloo)

UNIVERSITI TEKNOLOGI PETRONAS
BANDAR SERI ISKANDAR, PERAK
September 2016

CERTIFICATION OF ORIGINALITY

This is to certify that I am responsible for the work submitted in this project, that the original work is my own except as specified in the references and acknowledgements, and that the original work contained herein have not been undertaken or done by unspecified sources or persons.

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NUR SHAFIQA AQUILAH BINTI MAHMUD

ABSTRACT

This research aims to provide a measurement of the assessment of the water quality of polluted Penang River and to evaluate the seagrass bed in Penang Middle Bank's role as bio-indicator and phytoremediator to reduce heavy metals concentration in the water from being discharged further seawards. The river is containing of heavy metals from the discharge of land activities nearby the river. Thus, the heavy metals are harmful to the aquatic livings nearby the area. Seagrasses are great species for biomonitoring purposes which makes the seagrass bed very important to be preserved. The said seagrass bed is the second largest seagrass bed in Malaysia which is measuring of 50.6ha, home to various marine species such as turtles and dugongs, *sp. Enhalus Acoroides* (tape seagrass) and *sp. halophilia ovalis*, hermit crabs, clams, sea urchins, and octopus also comprising of at least six seagrass species. Samples of seagrass, sediment, and seawater were taken in September, October, and November 2016 and were analysed for its water pollution parameters and heavy metals accumulation. It is concluded that the water quality at the downstream of the Penang River improved seawards possibly due to the dilution of the river water by the seawater and none of the water quality parameters fall under class IV and V. Meanwhile for heavy metals analysis, the highest concentration of heavy metal is iron (Fe) with reading 4512.9 µg/g in sediment sample (S3) of September, then followed by chromium, manganese, zinc, copper, lead and cadmium. Seagrasses were observed to accumulate all of the tested heavy metals 60% more compared to sediment samples and 100% more compared to water samples.

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CHAPTER 1

INTRODUCTION

1.1 Background of Study

The study is to evaluate the existing pollution in the estuary of Penang River (*Sungai Pinang*) and the accumulation of metals at the seagrass bed in Penang Middle Bank (also known as *Pulau* Gazumbo), which is located between the river estuary of Penang River and the first Penang Bridge. Samples of aquatic plants (seagrass species), sediments, and water will be collected at the Middle Bank area. Middle Bank, Penang, is the second largest seagrass bed in Malaysia, measuring 50.6ha [1].

The seagrass bed is said to be the home of various marine species such as turtles and dugongs, tape seagrass and *halophilia*, hermit crabs, clams, sea urchins, and octopus. The bed is said to be comprising of at least six seagrass species [1].

Meanwhile, the Penang River is a seriously ill and polluted river, which its estuary is located near the Middle Bank with approximate distance of 800m. The river is amongst the seven most polluted river basins in Malaysia [2-3], almost all of the parameters tested on the water samples of Penang River fall down to class V of Interim National Water Quality Standards for Malaysia [4].

Seagrasses are a unique group of flowering plant that have adapted to exist fully submerged in the sea- profoundly influence the physical, chemical, and biological environments in coastal waters [5]. Seagrasses are generally threatened by anthropogenic influences [6] which the land use around the Penang River area includes textile and food industry, wet market, slaughter house, residential and commercial development while the river runs through a highly dense and populated area of Georgetown [4].

1.2 Problem Statement

The seriously ill and polluted Penang River is said to affect the health of marine lives in the coastal ecosystem. The Penang River water pollution is worsening from Class IV in 1999 [7] to Class V in 2013 [4]. In addition, the river is containing of heavy metals from the discharge of land activities nearby the river. Thus, the heavy metals are harmful to the aquatic livings nearby the area.

Additionally, the pollutions from the Penang River is thinning the Middle Bank seagrass bed that is located nearby the Penang River estuary. The thinning of the seagrass will affect the health of the seawater and aquatic living as the seagrass bed subsequently acts as a feasible bio-indicator in the coastal ecosystem near the river estuary and cleanse the water by absorbing dissolved metals.

1.3 Objectives

In pursuing this study, objectives below are to be achieved in order to counter the problem stated above.

1. To determine the water quality of the downstream of Penang River that is discharged to the coastal environment.
2. To evaluate the seagrass bed role as biomonitoring tool and phytoremediator in reducing heavy metals such as chromium (Cr), copper (Cu), manganese (Mn), Iron (Fe), and zinc (Zn).

1.4 Significance of Project

This research project is able to provide awareness in preserving the seagrass bed at Middle Bank from being degraded by emphasizing the roles of the seagrass bed. The presence of seagrass near the estuary of the polluted Penang River are able to reduce heavy metals concentration in the river water and seawater. This seagrass bed subsequently acts as a feasible bio-indicator in the coastal ecosystem near the river estuary and cleanse the water by absorbing dissolved metals.

1.5 Scope of Study

Water quality of the river water and seawater were analysed for biochemical oxygen demand (BOD), dissolved oxygen (DO), pH, temperature, turbidity, total suspended solids (TSS), total coliform (MPN), E.Coli (MPN), and ammoniacal-nitrogen (NH3-N). Meanwhile the heavy metals analysed were for cadmium, (Cd), chromium (Cr), copper (Cu), iron (Fe), manganese (Mn), lead (Pb), and zinc (Zn).

CHAPTER 2

LITERATURE REVIEW

2.1 Importance of Seagrass

Seagrass as one of the benthic species, are covering about 0.1% to 0.2% of the ocean floor and they are considered as a highly productive eco-system that plays a key role in the coastal zones [8]. They are the supplier of food and they act as nursey and shelter to various marine organisms which includes sea cucumbers, starfish, and seahorses [9-12]. Additionally, seagrasses influence the physical, chemical, and biological environments in coastal waters [5].

More reasons of seagrass bed represent one of the most important ecological components in the coastal ecosystem is that their leaves act as phytoremediators and cleanse seawater by absorbing dissolved metals [13] while their roots protects the shoreline by reducing erosion in occurrences of storms by gripping the seabed [14].

2.2 Polluted Penang River

The Penang River has been polluted and its water quality has been deteriorating for years, seriously affecting the environment and the ecosystem of the surrounding area of the river [4]. Almost all of the parameters tested on the water samples of Penang River fall down to class V of Interim National Water Quality Standards for Malaysia [4]. Despite the bad water quality, the Penang River water pollution is actually worsening from Class IV in 1999 [7] to Class V in 2013 [4].

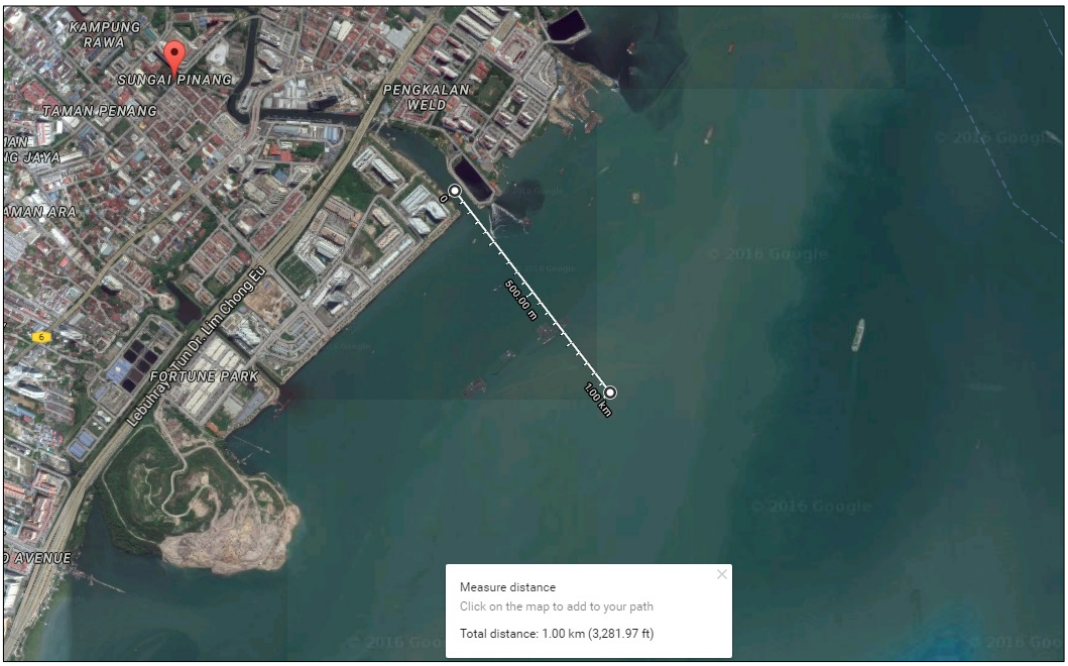

Figure 2.1 : Approximate distance of Middle Bank from Penang River Estuary (Source : Google Maps)

2.3 Seagrass as a Biological Indicator and Biomonitoring Tool

Biological indicator (bio-indicator) or biological monitor (biomonitor) for heavy metals is denoted as species which accumulates heavy metals in its tissues, and may therefore be analysed as a measure of the bioavailability of the metals in the ambient habitat [15]. To be able to select the right type of aquatic plants as bio-indicator, the species must be sedentary, of ecological importance, widespread as they are of approximately 60 species worldwide [6] and widely studied, sensitive to the environmental variations, act as the first stage in the food chain of the ecosystem, and are more rapid in the presence of pollutants compared to organisms living at higher stages [16]. Additionally, ideal biomonitors should also be easy to identify, abundant, long-lived, available for sampling throughout the year, and have sufficient tissue analysis [15].

Seagrasses are great bio-indicator as they integrate environmental impacts over measureable and definable timescales [17]. Additionally, seagrasses have high capacity to bind trace elements from the composition of their cellular wall, which is rich in hydroxyl, sulphate and carboxyl groups of polysaccharides

structures, and they act as important complexation site for metal and metalloid cations [18-19]. Despite that, they show extraordinary sensitivity to changes in water quality like changes in nutrients, organic matter, and turbidity, also to other human disturbances [20]. Leaves surfaces of seagrasses are able extract metals from water columns, while their roots extract metals from sediments and interstitial water [21].

2.4 Heavy Metals in Seagrass

Seagrass are used as bio-indicators and phytoremediators in measuring the concentration of heavy metal elements in the discharge of Penang River to the sea and its ability to remediate and cleanse the water passing. Leaves surfaces of seagrasses are able to extract metals from water columns, while their roots extract metals from sediments and interstitial water [21]. Based on Table 2.1, it is summarized that all of the stated seagrass species are bio-indicators for heavy metal elements of cadmium (Cd), copper (Cu), lead (Pb), and zinc (Zn).

Table 2.1 : Marine plant employed as bio-indicators of metallic contamination [25]

Species	Metals	References
Amphibolis Antarctica (Labill.) Sonder and Aschers. ex Aschers.	Cd, Cu, Fe, Mn, Pb, Zn	Harris et al. (1979)
Cymodocea nodosa	Cr, Cu, Ni; Al; Ca, Cd, Cu, Fe, K, Mg, Na, Pb, Zn	Catsiki and Panayotidis (1993), Malea (1993) and Malea and Haritonidis (1995)
Cymodocea rotundata Ehrenb. and Hempr. ex Aschers *C. serrulata* (R. Brown) Aschers. and Magnus *Enhalus acoroides* (L. f) Royle *Halodule uninervis* (Fors.) Aschers. *H. pinifolia* (Miki) den Hartog *Halophila ovalis* (R. Br.) Hook. f	Cd, Cu, Pb, Zn	Nienhuis (1986)
Halophila stipulacea (Fors.) Aschers.	Al; Cd, Cu, Fe, K, Na, Pb, Zn; Cd; Al	Malea and Haritonidis (1989b), Malea (1994a,b) and Malea and Haritonidis (1996)
Heterozostera tasmanica (Martins ex Aschers.) den Hartog	Cd, Cu, Fe, Mn, Pb, Zn; Cd	Harris et al. (1979) and Fabris et al. (1982)
Posidonia australis Hook. f	Cd, Cu, Mn, Ni, Pb, Zn	Ward (1987)
P. oceanica	Cd, Cr, Cu, Pb, Zn; Hg; all	Campanella et al. (2001) and Capiomont et al. (2000); see synthesis in Pergent-Martini and Pergent (2000)
Syringodium isoetifolium (Aschers.) Dandy *Thalassia hemprichii* (Ehrenb.) Aschers. *Thalassodendron ciliatum* (Forrsk.) den Hartog	Cd, Cu, Pb, Zn	Nienhuis (1986)
Zostera marina (L.)	Cd; Cd, Cu, Pb, Zn; Cd, Cu, Cr, Pb, Zn	Faraday and Churchill (1979), Brix et al. (1983) and Lyngby (1991)
Zostera muelleri Irmisch ex Aschers.	Cd, Cu, Fe, Mn, Pb, Zn; Cu	Harris et al. (1979) and Carter and Eriksen (1992)

2.5 Possible Interference from the Penang River Pollution to Seagrass Bed

The polluted Penang River is seen to be affecting the health of the seagrass bed. The seagrass bed at Middle Bank is thinning especially nearest to the Penang River Estuary. Besides having the seagrasses being stressed by the presence of heavy metals [25], there are other possible interferences that may have caused the thinning of the seagrass bed.

Amongst the problems that the seagrasses are facing due to the deterioration of the Penang River water quality are mainly interfering with the photosynthesis process of the seagrasses. The increased in turbidity of the Penang River water will cause a reduction in light penetration and limiting the depth range of the seagrass and sedimentation can smother seagrass or interfere with photosynthesis as the sediment settles [24]. Besides that, increased in nutrient loads in the water encourages algal blooms and epiphytic algae to grow to a point where it smothers or shades seagrasses, also reducing photosynthetic capacity [24]. Also, the herbicides that flows into the water from land activities can kill seagrasses and other chemicals can kill associated macro-fauna in the area [24].

CHAPTER 3

METHODOLOGY

3.1 Location and Sampling Method

In this chapter, the sample collections, preparations, and analyses are discussed in details. The location of the study which is the Middle Bank in Penang (Figure 3), were analysed thoroughly and the condition of the area were observed. Four sampling points were finalized (as shown in Figure 3) starting from the downstream of Penang River towards the sea, labelled as point 1, 2, 3, and 4 as detailed in Table 3.1.

Table 3.1 : Details of sampling points and samples collected

Sampling Points	Coordinate	Sample	Name
1 Downstream of Penang River	N 05.40429°, E 100.32793°	River water	W 1
2 Estuary of Penang River	N 05.40429°, E 100.32793°	River water	W 2
3 Middle Bank seagrass bed	N 05.39668°, E100.33918°	Seawater	W 3
		Sediment	S 3
		Seagrass	G 3
4 Pulau Besar (further from Penang River discharge)	N 05.36457°, E 100.32665°	Seawater	W 4
		Sediment	S 4
		Seagrass	G 4

Additionally, existing data of the environmental quality were obtained to be able to have a comparison with this research study and as an overview of the latest condition of the location. Samples of sediment, seagrass, and water (seawater and river water) were collected in the month of September, October, and November of 2016. These samples were experimented and analysed. The samples were collected during low tide phase of every month to be able to reach the seabed surface. The research process flow is as summarized in Figure 3.1, detailed in Figure 3.1.1 and Figure 3.1.2 below.

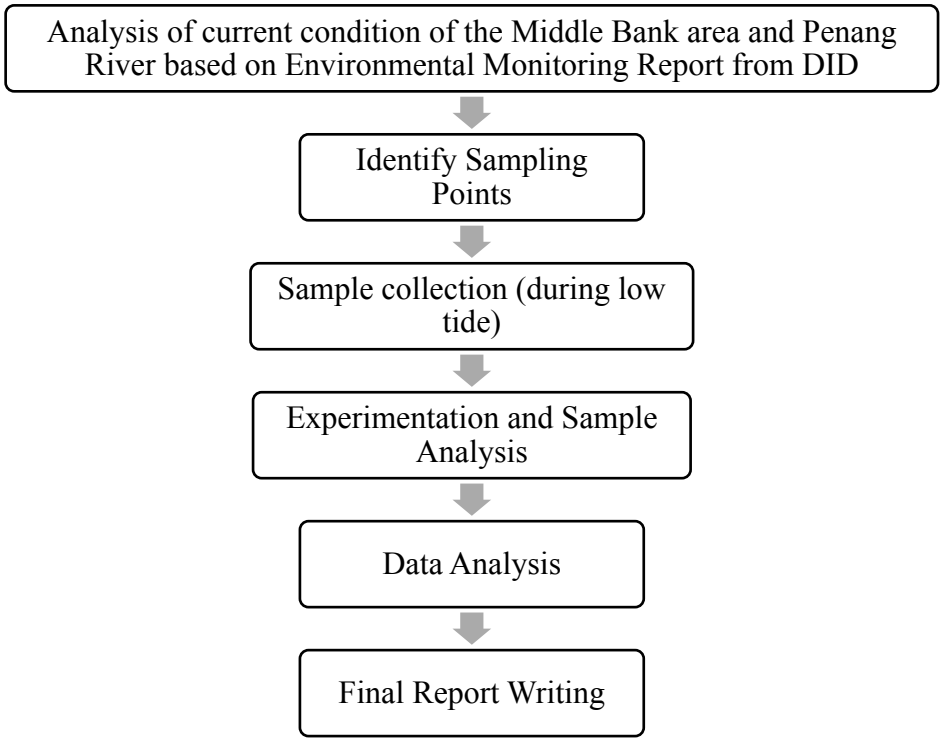

Figure 3.2 : General Flowchart of overall research process

3.2 General Flow of Experimental Method

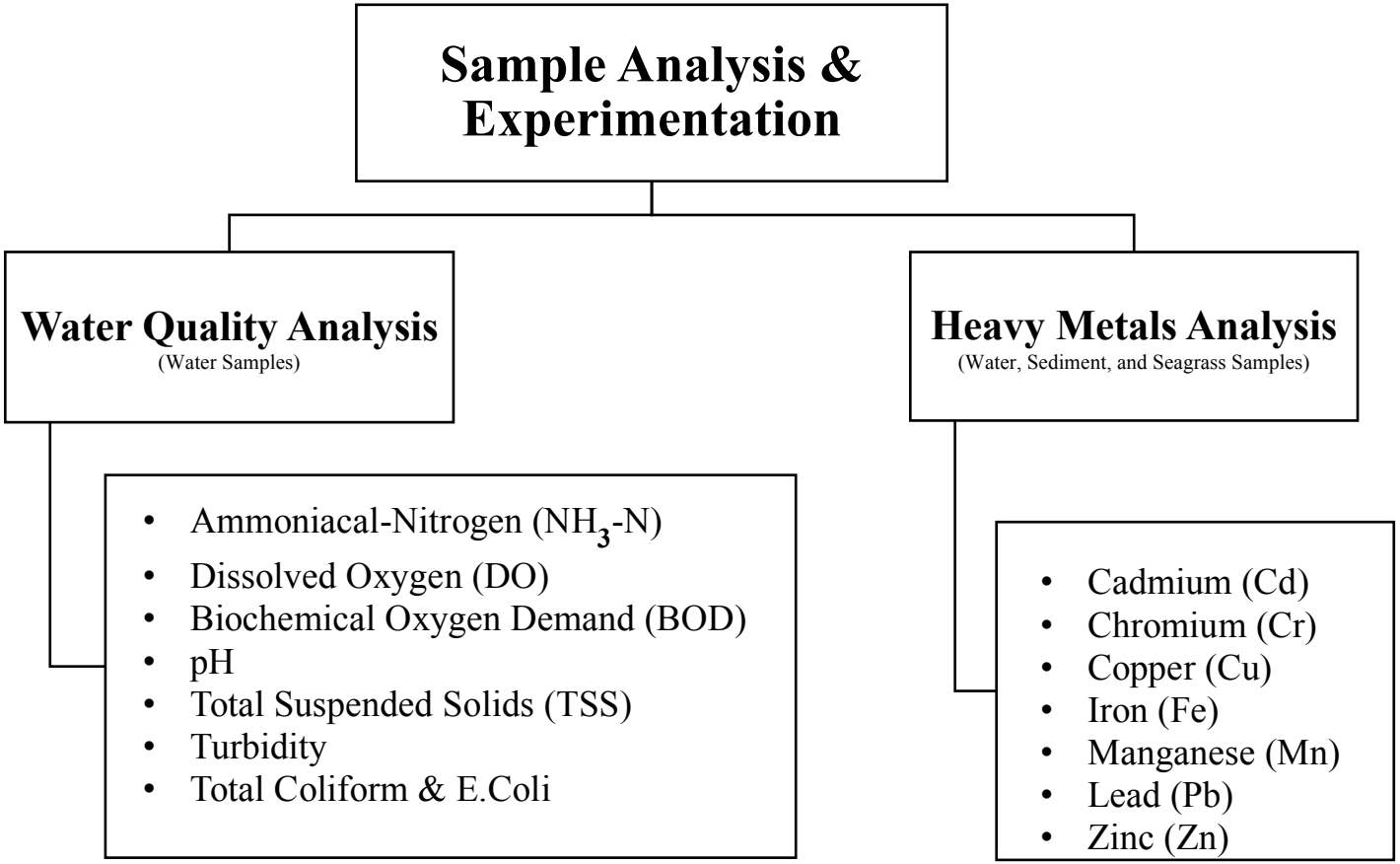

Figure 3.1.1 : Flow of Sample Analysis

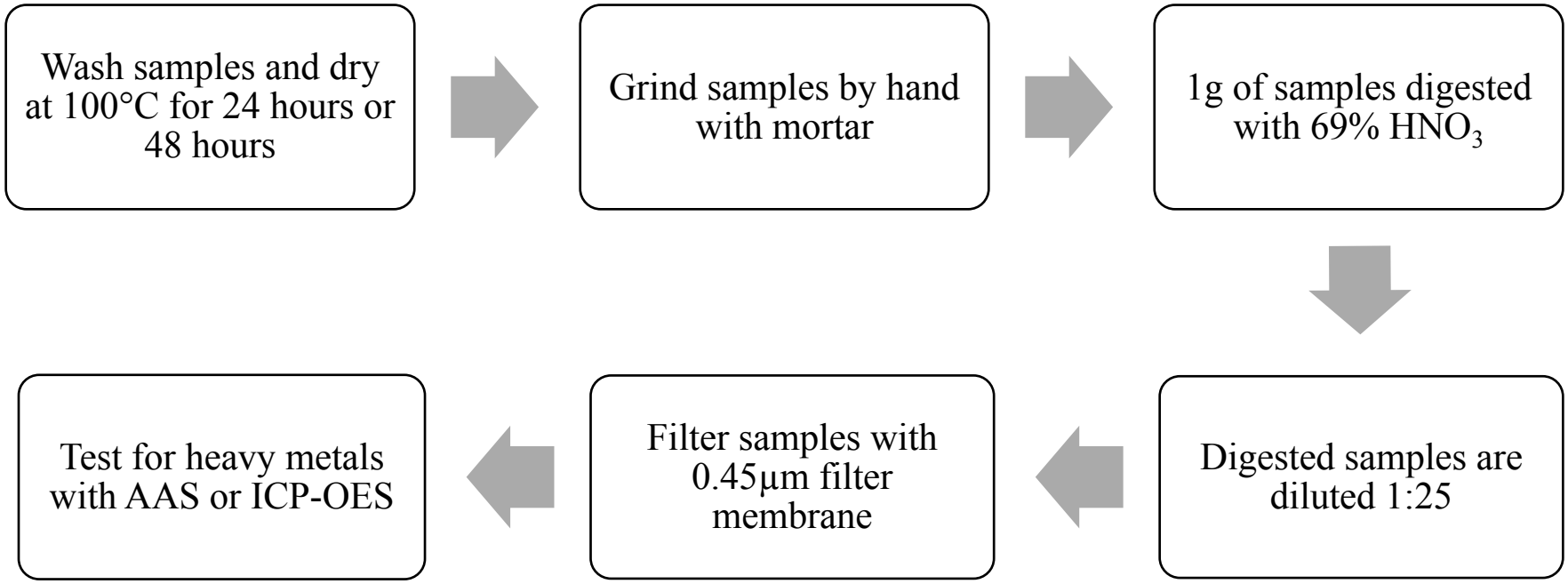

Figure 3.1.2 : Flow of Tissue Digestion Process for Sediment and Seagrass Samples for Heavy Metals Analysis.

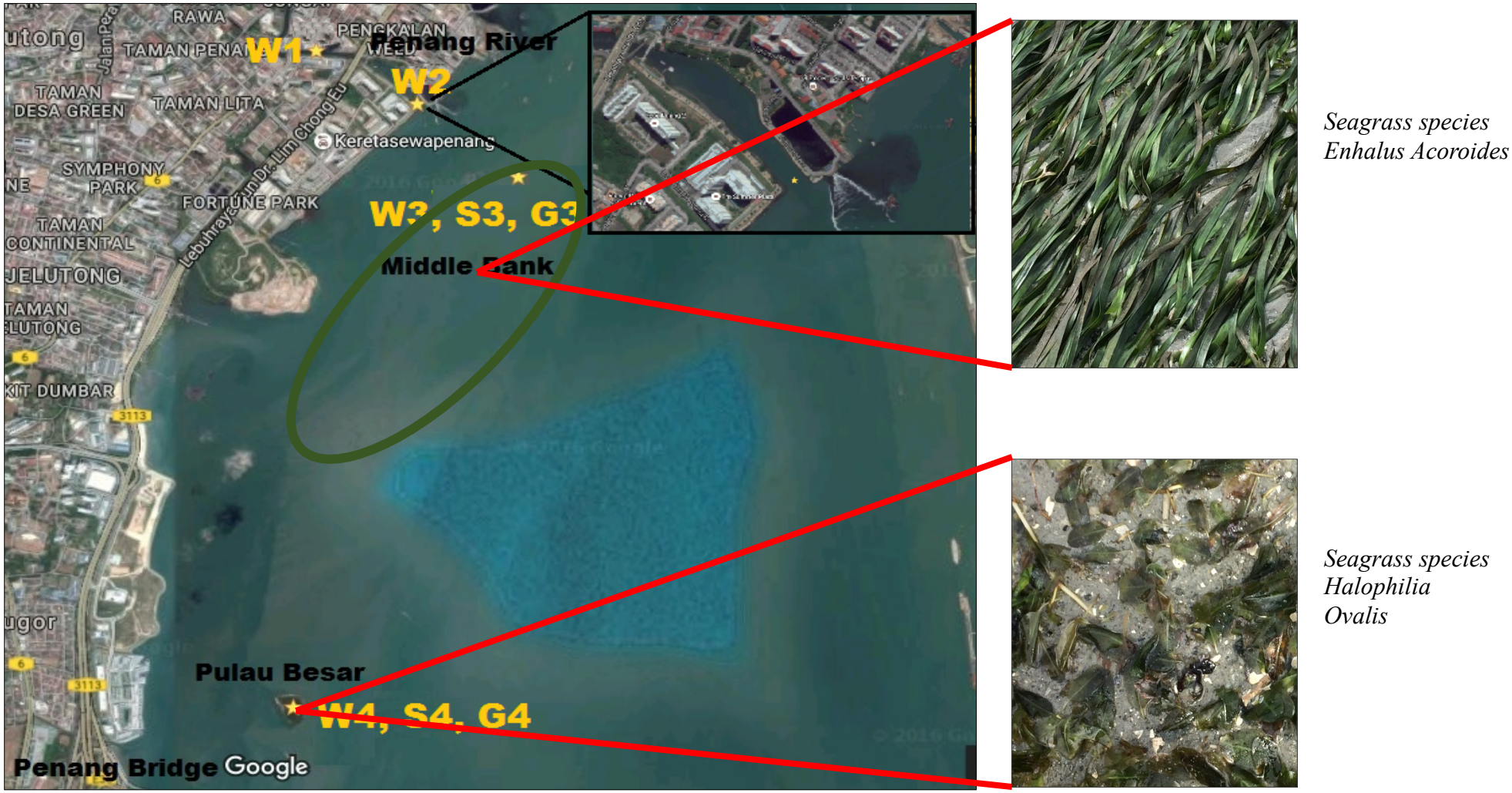

Figure 3.2 : Sampling Points and Seagrass Species Available

3.3 Water Samples Preparation and Analysis

Other than that, water samples from each points are to be taken and stored at 4°C, taken too with its temperature. The water samples will be analysed for its ammoniacal nitrogen (NH_3-N), biochemical oxygen demand (BOD), dissolved oxygen (DO), pH, total suspended solids (TSS), turbidity, total coliform and *E.coli* contents. Meanwhile for heavy metals analysis, water samples are analysed for Chromium (Cr), Copper (Cu), Iron (Fe), Manganese (Mn), and Zinc (Zn). All analyses are done in triplicates.

3.3.1 Ammoniacal-Nitrogen (NH_3-N) Analysis

The samples are tested for its ammoniacal-nitrogen using Nessler Method. 25-mL of sample is prepared in a mixing cylinder, added with three drops of mineral stabilizer and inverted several times. Then, three drops of Polyvinyl Alcohol Dispersing Agent are added into the mix and also inverted several times to mix. 1.0mL of Nessler Reagent is then pipetted into the mix and inverted several times, followed by a one-minute reaction period. 10mL of the mix is transferred into square sample squares. The same procedure is repeated for each sample. Before the samples are read with DR 5000 Spectrometer, a blank is prepared in the same method instead of water sample but only deionized water.

Note : the samples are to be diluted with appropriate dilution factor when necessary if the concentration of NH_3-N is expected to be high or if the spectrometer reading is out of range.

3.3.2 DO Analysis

Using a portable DO probe, the DO reading of the seawater and river water were taken directly on site.

3.3.3 Biochemical Oxygen Demand (BOD)

300mL of samples (diluted with aerated distilled water) from each sampling points were taken and transferred to BOD bottles. The sample volumes used are 100mL. Before analysing, the DO meter is calibrated and the initial DO reading is taken.

After the initial DO was recorded, the BOD bottles with samples are placed in BOD refrigerator with 20°C temperature. The samples are refrigerated for 5 days, to analyse for BOD_5. After 5 days, the final DO reading is taken again using DO meter. The value of BOD (mg/L) is calculated as below :

$$BOD\ (\frac{mg}{L}) = \frac{(initial\ DO - final\ DO - blank\ correction) * 300mL}{volume\ of\ sample} \quad (1)$$

3.3.4 pH Analysis

100mL of water samples were taken from the collected samples and are transferred to small beakers. pH mater is to be calibrated before being placed in the beaker to determine the pH readings of the samples.

3.3.5 Total Suspended Solids

The total suspended solids (TSS) of each water samples are experimented using gravimetric method. Initial weights of filter papers are recorded. 100mL of well-mixed water samples are pumped and filtered with 47mm sized filter paper. After all the water samples are filtered, the filter discs with the solids are dried at 103°C for 1 hour. After drying, the filter discs are left to cool in room temperature in a desiccator, then measured for its final weight. The TSS value is calculated in mg/L as below :

$$TSS\ (\frac{mg}{L}) = \frac{(weight\ of\ filter\ discs\ after\ drying) - (weight\ of\ filter\ discs\ before\ drying)}{sample\ size\ in\ L} \quad (2)$$

3.3.6 Turbidity Analysis

10mL of water samples shall be prepared, transferred into sample cells up to its markings. The sample cells are placed in the turbidimeter to measure its turbidity values.

3.3.7 Total Coliform and E.Coli Analysis

Most Probable Number (MPN) is to be used to determine the measurement of total *coliform* and *E. Coli.* 100mL of samples were prepared added with Colilert reagent, transferred into IDEXX Quanti-Tray 2000 and sealed. The trays are then to be placed inside an incubator for a 24-hour observation. The colours are observed for presence of *Coliform*. Meanwhile for *E.Coli*, the samples are to observed under UV light and record the positive cells. The MPN values are determined using the IDEXX Quanti-Tray 2000 MPN Table for 100mL sample.

3.3.8 Heavy Metals Analysis for water samples

i. Chromium (Cr)

Water samples are analysed for Chromium concentration using 1,5-Diphenylcarbohydrazide Method[1] with powder pillows. 10mL of sample is filled into a sample cell, added with one ChromaVer® 3 Reagent Powder Pillow to the sample cell. The sample cell is swirled to mix the solution, and the solution is left for a 5-minute reaction period. A blank is prepared by filling another sample cell with 10mL sample without adding any powder pillow. After zeroing the spectrometer, the sample solution with powder pillow is read.

ii. Copper (Cu)

Water samples are analysed for Copper concentration using bicinchoninate method[1] with powder pillows. 10mL of sample is filled into a sample cell, added with one CuVer® 1 Copper Reagent Powder Pillow to the sample cell. The sample cell is swirled to mix the solution, and the solution is left for a 2-minute reaction period.

A blank is prepared by filling another sample cell with 10mL sample without adding any powder pillow. After zeroing the spectrometer, the sample solution with powder pillow is read.

iii. Zinc (Zn)

Water samples are analysed for Zinc concentration using Zincon method[1] with powder pillows. 20mL of sample is filled into a mixing cylinder, added with one ZincoVer® 5 Reagent Powder Pillow to the cylinder. The cylinder is inverted several times, then pour 10mL solution into a square sample cell as blank. 0.5mL of cyclohexane solution into the remaining sample in the cylinder, the solution is left for a 30-second reaction period. During the reaction, the cylinder is stoppered and shaken vigorously, then the solution is left for a 3-minute reaction period. 10mL of the prepared solution is poured into another sample cell. After zeroing the spectrometer with the prepared blank, the second sample cell is read for zinc concentration.

iv. Iron (Fe), Manganese (Mn), and Zinc (Zn)

Meanwhile for water samples that are to be analysed for Fe, Mn, and Zn, these samples are filtered directly with a 0.45µm filter membrane. The samples are sent for Absorption Spectrometer Model Analyst using flame atomizer.

3.4 Sediment and Seagrass Preparation and Analysis

Meanwhile, for aquatic plants (specifically seagrass) samples, they are to be collected with a scoop and kept in a clean and sealed plastic bags, together with roots, rhizomes, and leaves. They are then to be washed with seawater to remove sediments [34]. The seagrass samples will also be stored the same way as the sediment samples are stored, refrigerated at 4°C. Samples of seagrass are to be washed again to remove any excessive epiphytes then are dried at 100°C for 24 to 48 hours until a constant weight is reached.

To have the samples to be in homogeneous powder, they are to be grinded using agate mortar. The powdered samples are then digested by having 1g of the dried

samples with 10mL of nitric acid, HNO_3. The samples are stirred and heated at 100-120°C. Hydrogen Peroxide, H_2O_2, is added in small volume (1mL) to catalyse the digestion process. 8mL of HNO_3 is added before the solution is dried out to ensure that all of the samples are thoroughly digested, and H_2O_2 is too added to catalyse the process; this method is repeated until all of the sample is in liquid form.

1mL of the digested solution is left cooled, and is diluted with distilled water up to 25mL solution. The solution is filtered with 0.45µm filter membrane. The filtered solutions are then tested for Chromium (Cr), Copper (Cu), Iron (Fe), Manganese (Mn), and Zinc (Zn) concentration using Absorption Spectrometer Model Analyst using flame atomizer.

3.5 Project Milestones and Timeline

In completing this study, key milestones and timeline planned is as follows.

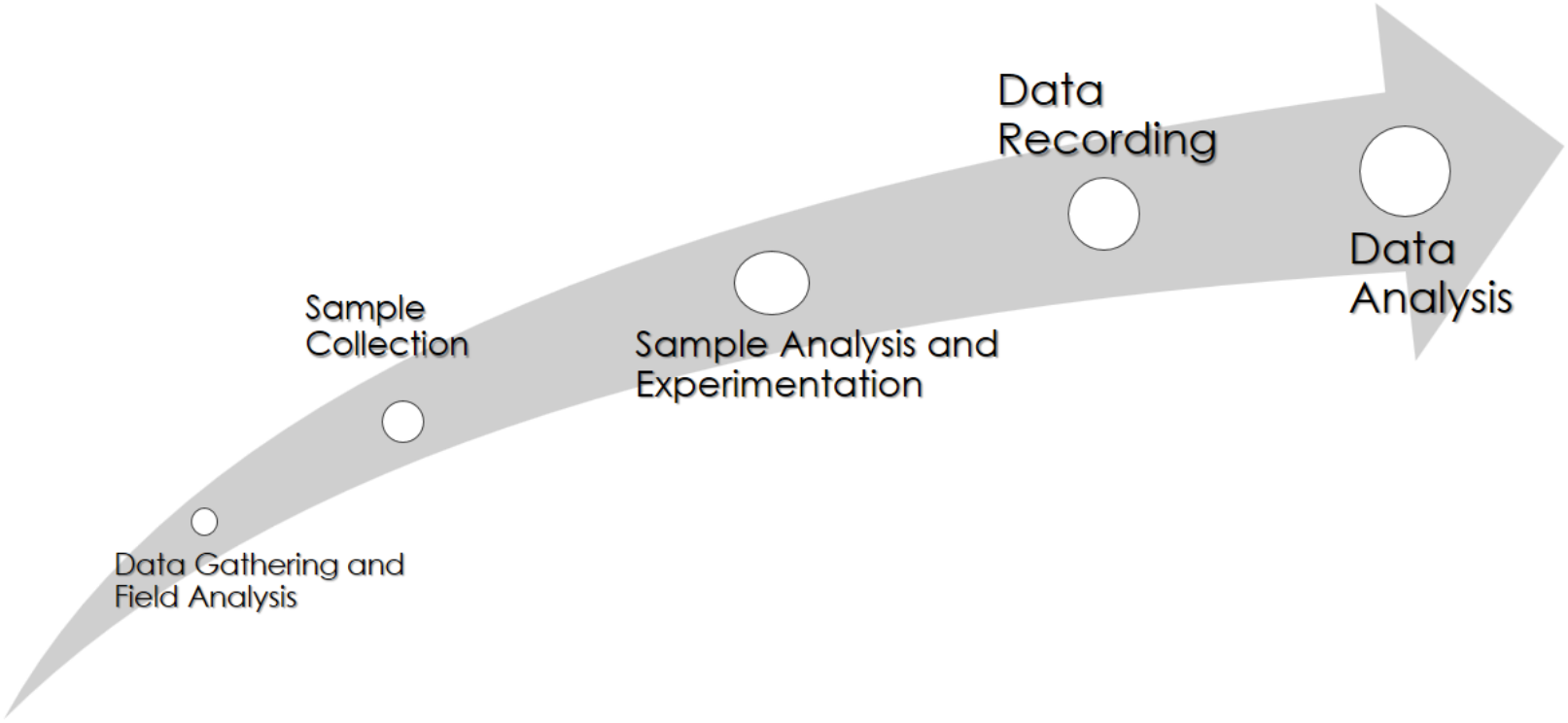

Figure 3.3 : Key Milestone

No.	Detail \ Week	FYP 1 : Week														FYP 2 : Week													
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	Data Gathering and Field Analysis																												
2	Submission of Extended Proposal																												
3	Proposal Defense																												
4	Sample Collection																												
5	Sample Analysis and Experimentation																												
6	Data Analysis																												
7	Preparation of Interim Report																												
8	Preparation of Progress Report																												
9	Draft of Final Report																												
10	Preparation of Dissertation																												
11	Preparation of Technical Paper																												
12	Preparation for Viva																												

Figure 3.4 : Gantt-Chart of Project Timeline

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Site Observation

Sampling were done three times throughout the study, which was in September, October, and November. Table 4.1 below shows the weather and tide conditions during the 3-months sampling.

Table 4.1 : Weather and tidal conditions during sampling

Sampling Date	Weather	Tide
15th September 2016	Drizzling	Low tide but water started flooding
18th October 2016	Clear but raining the night before	Low tide, water slightly flooded
18th November 2016	Clear but raining the night before	Low tide

4.2 Water Quality Analysis

Below is the comparison of water quality parameters during 3-months sampling period. Referring to Table 4.1 and Figure 4.1 – 4.7, the water quality classes are identified in accordance to Interim National Water Quality Standards (INWQS) for Malaysia. The major differences of data in September, October, and November possibly influenced by the different tidal conditions and weather conditions.

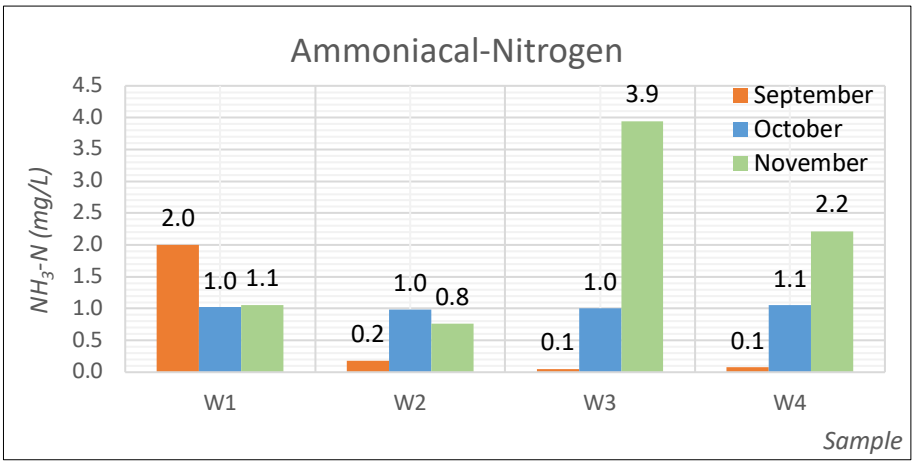

Figure 4.1 : Ammoniacal-Nitrogen concentration in water samples in the months of September to November 2016.

NH3-N concentration was 2.0mg/L, which was highest at the river and improved seawards in September while the value was constant in October possibly due to the tidal changes and movement of the seawater during sampling. Meanwhile in November, it showed a different pattern, as the value is highest at point 3. The highest concentration recorded was 3.94 mg/L in November at point 3. The high concentration of NH3-N at point 3 (Middle Bank) may be contributed by the fish farm that is located about 500m from Point 3 which is at the Middle Bank.

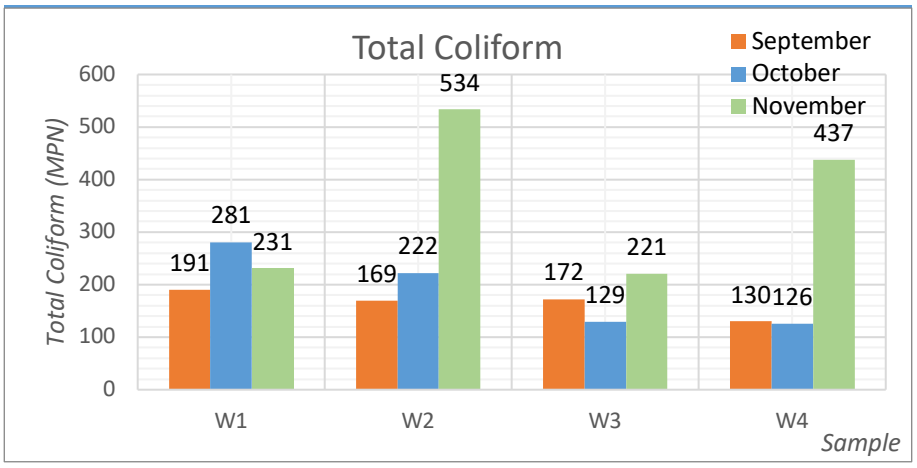

Figure 4.2 : Total Coliform concentration in water samples in the months of September to November 2016.

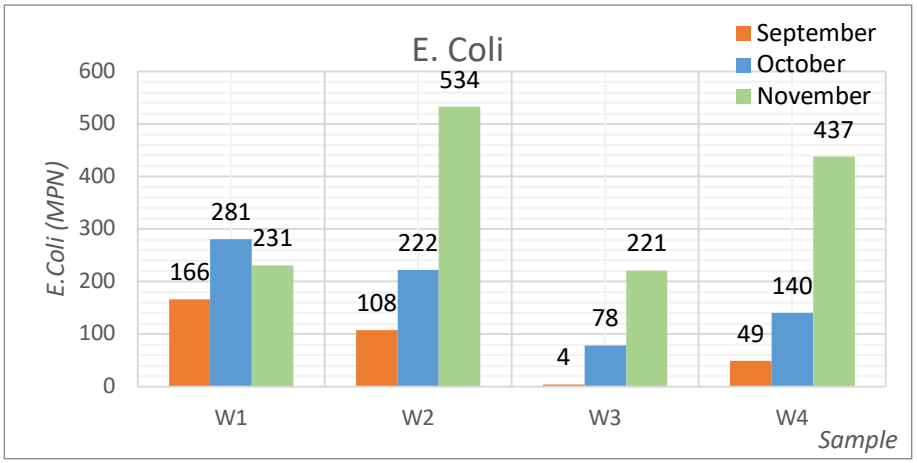

Figure 4.3 : E.Coli concentration in water samples in the months of September to November 2016.

Total coliform showed a similar pattern in September and October which the values are reducing seawards as in Figure 4. While in November, the bacterial reading was highest at point 2, which is at the estuary of Penang River. This can be justified due to the presence of stray dogs around the land area while sampling near the estuary during the low tide. Presence of the stray dogs may indicate that there were sources of food for them to feed on (i.e. carcasses and food waste) which contributed to bacterial formation at point 2. Additionally, another possible reasons of the high concentration of total coliform at point 2 is may due to a discharge of sewage water from a sewage treatment plant beside the river estuary while sampling.

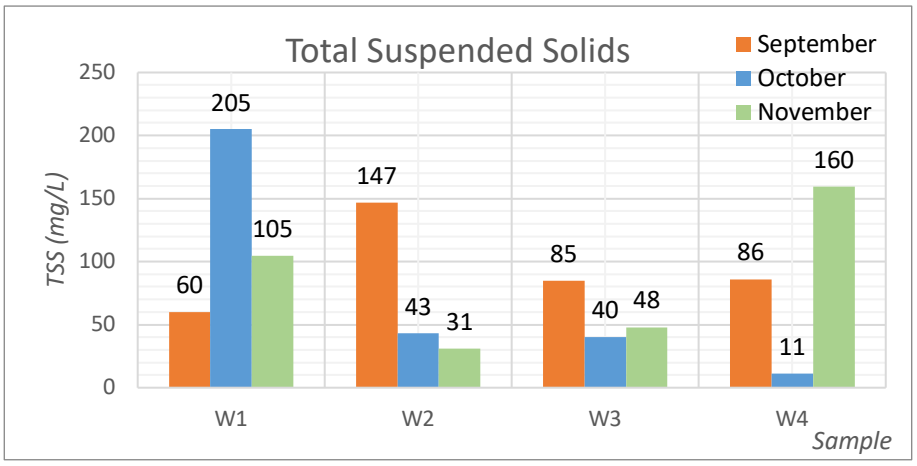

Figure 4.4 : Total suspended solids in water samples in the months of September to November 2016.

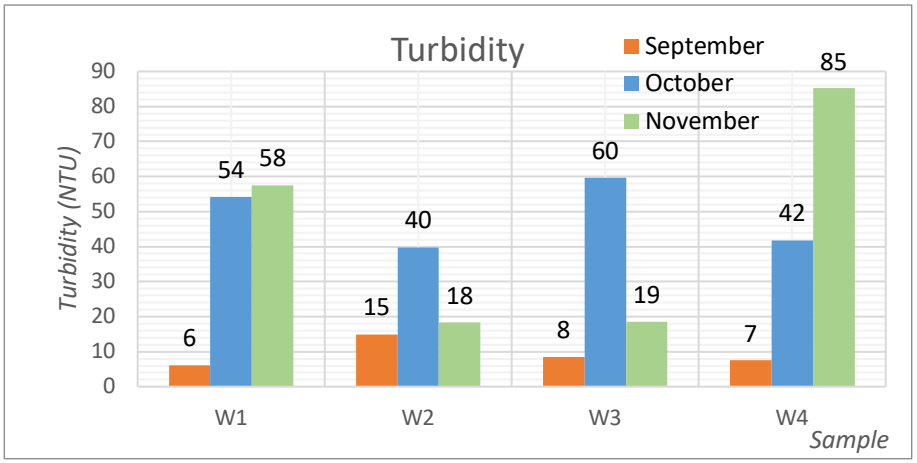

Figure 4.5 : Turbidity concentration in water samples in the months of September to November 2016.

TSS values in September varied but as the water left the estuary, it showed improvements. While in October and November, a clear pattern that the water was improving seawards was observed. TSS values are related with turbidity readings, hence turbidity showed a similar pattern. The major differences of Turbidity for September and October was possibly due to the different tidal conditions. The reduction of TSS readings seawards may be due to the dilution of the river water as it is discharged to the sea. Although so, a higher reading of TSS at point 3 in October, and at point 4 in November may be affected by the suspended sediments in the water as the water samples were taken at shallow water depth at both points. The water at point 4 were seen to be muddy, hence resulting to high TSS values.

Relating the high total coliform value in Figure 4 and high TSS values in Figure 5 for the month of November at point 4, this can be deduced that the suspended solids were also contributing to the bacterial formation. One of the possible reasons of this is that there may be dumping of materials (i.e. rubbish, contaminated water or material, fish waste or other farmed livings.) in the area that has contributed to such increment of values.

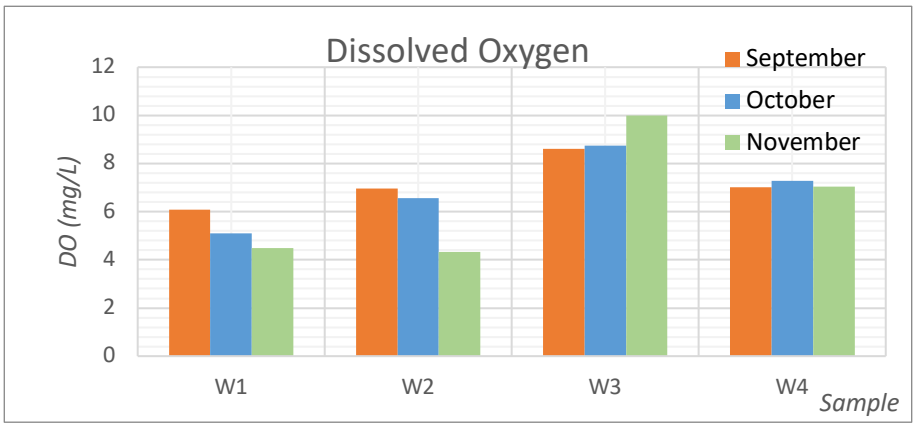

Figure 4.6 : Turbidity concentration in water samples in the months of September to November 2016.

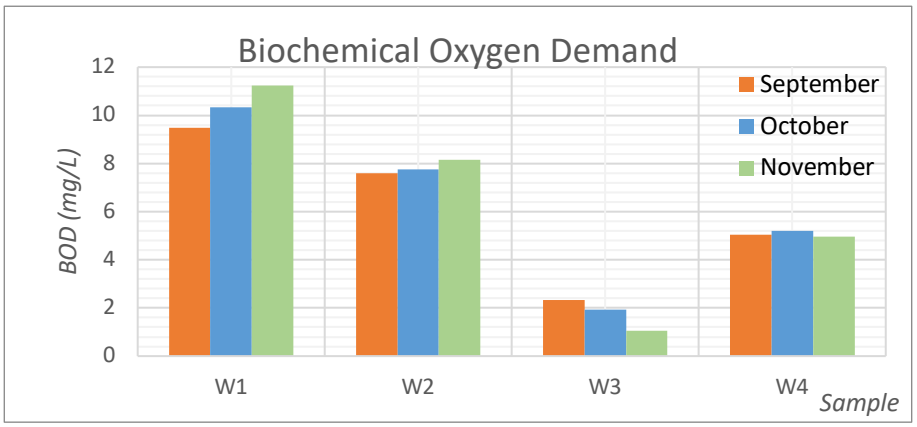

Figure 4.7 : Turbidity concentration in water samples in the months of September to November 2016.

The DO values for all the water samples are above 4.0 mg/L. DO and BOD were at class II and class III in the river and showed improvement to Class I seawards at point 3. Then at point 4, DO and BOD was class I and class II. BOD showed highest values at point 1 and 2 compared to point 3 and 4. This showed that there is a higher amount of oxygen used at point 1 and 2, indicating

that more possibilities of aerobic bacteria are present at these points. Higher BOD values indicates that the water is more polluted. This is seen as the BOD values at point 1 and 2 are higher and they are more polluted compared to point 3 and 4. Meanwhile, W1 and W2 showed lower DO readings compared to W3 (seagrass bed) and W4 (Pulau Besar). This indicates that the seagrass bed is functioning as an important habitat for other marine livings since the high DO concentration is crucial for their survival.

4.3 Heavy Metals Analysis

Below is the comparison of heavy metals analysis during 3-months sampling period.

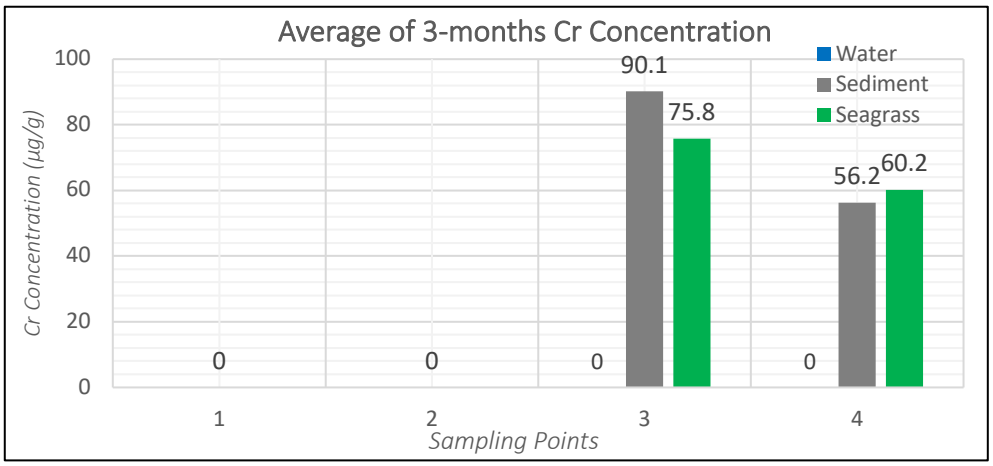

Figure 4.8 : Chromium concentration in water, sediment, and seagrass samples in the months of September to November 2016.

Sp. Enhalus Acoroides (G3) accumulated more chromium element than *sp. Halophilia Ovalis* (G4) by 21% but lesser than S3 by 16% at point 3. Meanwhile, *sp. Halophilia Ovalis* (G4) accumulated more chromium element than S4 at point 4 by 6.6%.

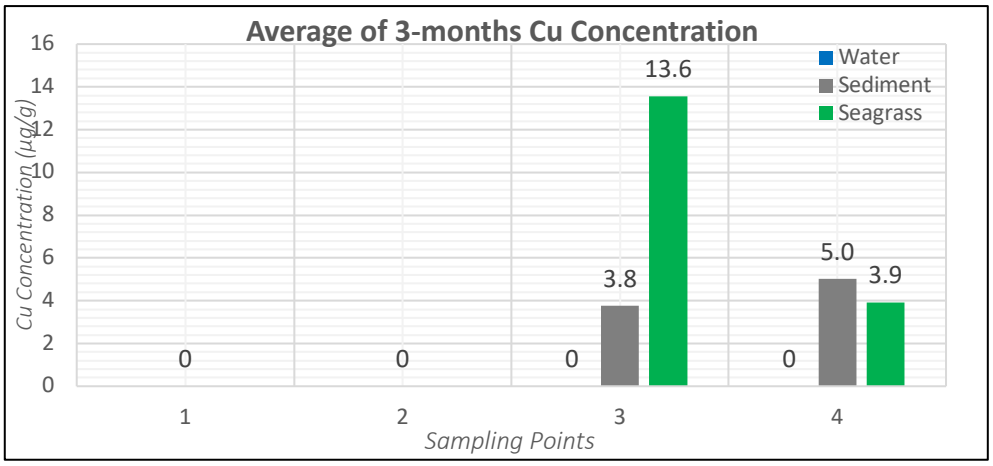

Figure 4.9 : Copper concentration in water, sediment, and seagrass samples in the months of September to November 2016.

Sp. Enhalus Acoroides (G3) accumulated more copper element than *sp. Halophilia Ovalis* (G4) by 71%. Meanwhile, *sp. Enhalus* Acoroides (G3) accumulates 72% more copper element than S3.

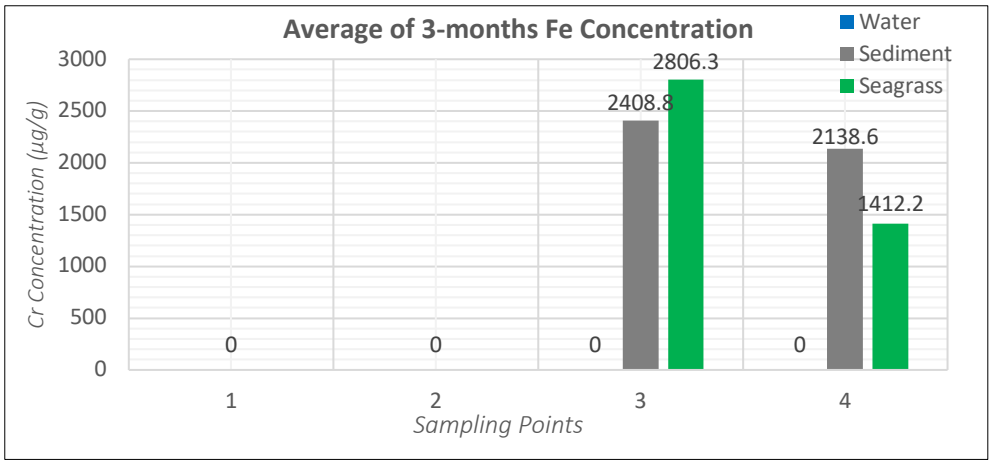

Figure 4.10 : Iron concentration in water, sediment, and seagrass samples in the months of September to November 2016.

Sp. Enhalus Acoroides (G3) accumulated more iron element than *sp. Halophilia Ovalis* (G4) by 50%, and 14% more accumulation compared to in sediment sample (S3).

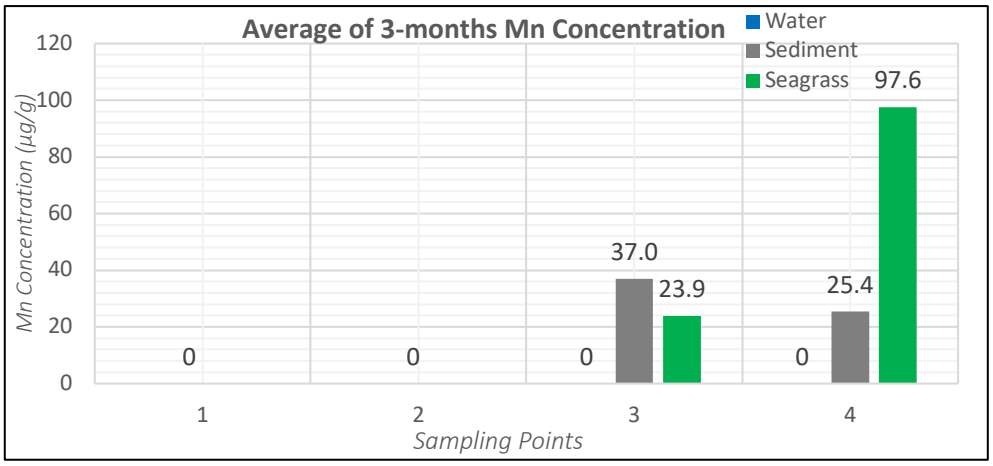

Figure 4.11 : Manganese concentration in water, sediment, and seagrass samples in the months of September to November 2016.

Sp. Halophilia Ovalis (G4) showed 75% more concentration of manganese element compared to S4. Meanwhile, *sp. Halophilia Ovalis* (G4) accumulated more manganese element than *sp. Enhalus Acoroides* (G3) by 74%.

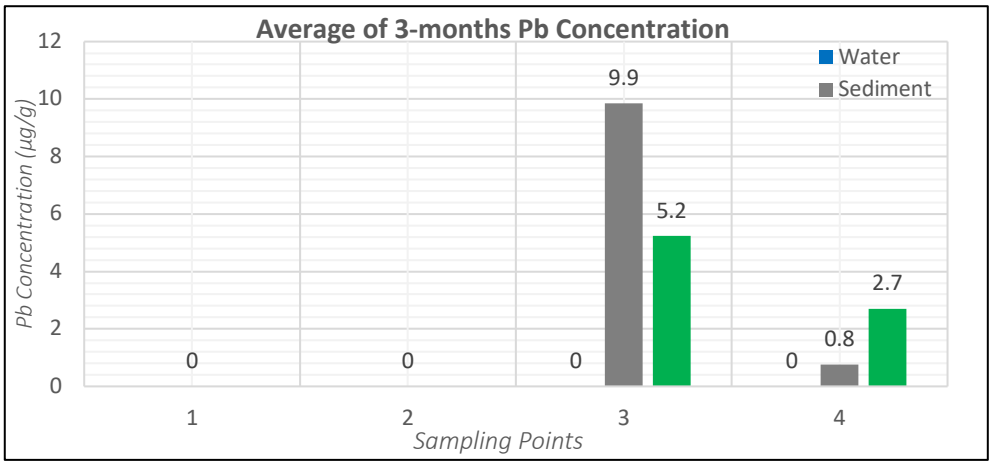

Figure 4.12 : Lead concentration in water, sediment, and seagrass samples in the months of September to November 2016.

Sp. Halophilia Ovalis (G4) showed 70% more concentration of lead element compared to S4. Meanwhile, *sp. Enhalus Acoroides* (G3) accumulated more lead element than *sp. Halophilia Ovalis* (G4) by 48%.

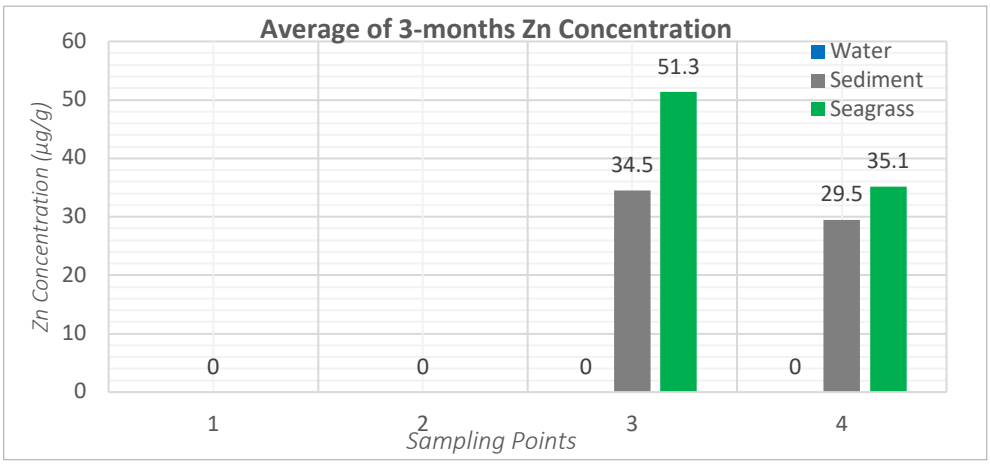

Figure 4.13 : Zinc concentration in water, sediment, and seagrass samples in the months of September to November 2016.

Both seagrass species (G3 & G4) accumulated more of zinc element compared to sediment (S3 & S4) by 33% and 16%. Meanwhile, *Sp. Enhalus Acoroides* (G3) accumulated more zinc element than *sp. Halophilia Ovalis* (G4) by 32%.

4.3.1 Summary of Heavy Metals Analysis

Referring to Figure 4.8 until 4.13, the highest concentration of heavy metal is Iron (Fe) with reading 4512.9 µg/g in S3 of September. After Iron as the highest heavy metal concentration, then comes chroumium (Cr), manganese (Mn), zinc (Zn), copper (Cu), lead (Pb), and cadmium (Cd), whereby Fe > Cr > Mn > Zn > Cu > Pb > Cd.

Overall, out of all the samples for, sediment and seagrass, it showed that seagrass shows a higher reading of 60% more heavy metals accumulation compared to sediment, and seagrass showed 100% more concentration that water samples.

Additionally, for heavy metals accumulation in seagrass, it is observed that both of the seagrass species are accumulating all of the tested heavy metals except cadmium (Cd). This can be deduced that there are no Cadmium elements in the water body and the polluted river.

Meanwhile, it is observed that sp. Halophilia Ovalis has the ability to absorb more of manganese element compared to sp. Enhalus Acoroides.

This can be seen as the manganese concentration in sediment samples, S3 and S4, has only slight difference, while the concentration of manganese in sp. Halophilia Ovalis is significantly higher than in sp. Enhalus Acoroides.

During site visits, it was observed that *sp. Enhalus Acoroides* was abundant at the Middle Bank (point 3) and only very *little sp. Halophilia Ovalis* was present at the area. While at *Pulau Besar* (point 4) which is approximately 3.4km from Middle Bank, only *sp. Halophilia Ovalis* was abundant as observed. *Sp. Halophilia Ovalis* indicates absenteeism when in stressed by heavy metals Cd, Cu, Pb, and Zn [22]. The absenteeism of *sp. Halophilia Ovalis* at the Middle Bank indicated that the area is prone to Cd, Cu, Pb, and Zn heavy metals pollutants. Also, the thinning of the seagrass bed nearest to the river mouth also showed that the seagrasses are under stressed especially nearest to the river estuary.

CHAPTER 5

CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

The water quality at the downstream of the Penang River improves seawards. None of the water quality parameters fall under class IV and V. Also, the water quality of the downstream Penang River showed improvements seawards, possibly due to the dilution of the Penang River water by seawater.

Meanwhile, seagrass samples were observed to be accumulating all of the tested heavy metals significantly as compared to sediment samples and water samples. The highest accumulation of heavy metals in the seagrasses is iron (μg/g), followed by chromium (75.8 μg/g), manganese (97.6 μg/g), zinc (51.3 μg/g), copper (13.6 μg/g), lead (5.2 μg/g), and cadmium (0.0 μg/g) The accumulation of heavy metals by these seagrasses (*sp. Enhalus Acoroides* at Middle Bank, and *sp. Halophilia Ovalis* at *Pulau Besar*) is crucial as they act as bio-indicator to represent the marine water condition also as phytoremediator in reducing the heavy metals pollutants from the discharge of the Penang River from going further seawards.

5.2 Recommendation

Throughout completing this project, the author has encountered a few steps that can be improved in order to have a better research results. During sampling, it is better to choose a sampling date (in low tide) with the most similar weather conditions with one sampling batch and another. This is because the difference in the weather conditions (i.e. rain) will affect the water quality parameters concentrations in the samples as the water is diluted by the rain water.

Meanwhile, for further research, more species or aquatic livings can be collected to be analysed (i.e. seashells, mussels, etc.). By analysing a wider variety of species, a clearer significance of the seagrass as biomonitoring tool and phytoremediators can be established.

Also, more heavy metals heavy (i.e. mercury, arsenic, aluminium, nickel) metals parameters can be analysed to better emphasizing the ability of seagrasses in accumulating heavy metals compared to other aquatic livings.

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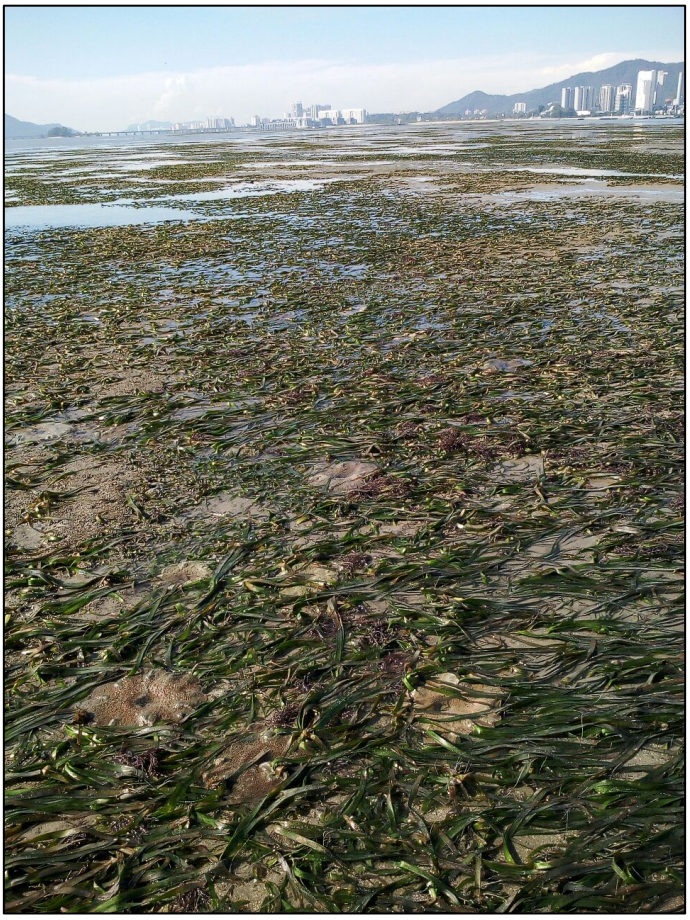
APPENDICES

Map view of Penang

Seagrass bed during low tide at the Middle Bank during sampling in November 2016



Seagrass *sp. Enhalus Acoroides* seen at Middle Bank during low tide in November 2016

View of the sides of Penang River

View of the port of Penang River

Seagrass bed is thinning nearest to the Penang River estuary as seen in October 2016.

Water Quality Analysis data in September, October, and November 2016

Parameters	Sampling Batch		
	Sep (A)	Oct (B)	Nov (C)
River Water at Point 1 – W1			
Ammoniacal-Nitrogen (mg/L)	2.0	1.0	1.1
BOD (mg/L)	9.5	10.3	11.2
DO (mg/L)	6.1	5.1	4.5
Total Coliform (MPN)	185.8	280.9	231.0
E. Coli (MPN)	301.5	280.9	231.0
TSS (mg/L)	64.0	205.0	105.0
Turbidity (mg/L)	6.1	54.2	57.5
pH	6.8	6.5	6.5
River Water at Point 2 – W2			
Ammoniacal-Nitrogen (mg/L)	0.2	1.0	0.8
BOD (mg/L)	7.6	7.8	8.2
DO (mg/L)	7.0	6.6	4.3
Total Coliform (MPN)	169.3	222.1	533.5
E. Coli (MPN)	107.8	222.1	533.5
TSS (mg/L)	147.0	43.0	31.0
Turbidity (mg/L)	14.9	39.8	18.3
pH	7.8	6.6	6.6
Seawater at Point 3 – W3			
Ammoniacal-Nitrogen (mg/L)	0.1	1.0	3.9
BOD (mg/L)	2.3	1.9	1.1
DO (mg/L)	8.6	8.7	10.0
Total Coliform (MPN)	171.7	129.4	221.2
E. Coli (MPN)	4.2	78.1	221.2
TSS (mg/L)	85.0	40.0	48.0
Turbidity (mg/L)	8.5	59.6	18.5
pH	7.9	8.0	7.8
Seawater at Point 4 – W4			
Ammoniacal-Nitrogen (mg/L)	0.1	1.1	2.2
BOD (mg/L)	5.0	5.2	5.0
DO (mg/L)	7.0	7.3	7.1
Total Coliform (MPN)	129.9	125.5	437.4
E. Coli (MPN)	49.4	140.2	437.4
TSS (mg/L)	86.0	11.0	160.0
Turbidity (mg/L)	7.5	41.8	85.2
pH	7.9	8.0	8.8

Heavy Metals concentration data in sediment samples in September, October, and November 2016

Parameters (mg/L)	Sampling Batch		
	Sep	Oct	Nov
Sediment at Point 3 – S3			
Cadmium, Cd (µg/g)	0.0	0.0	0.0
Chromium, Cr (µg/g)	215.5	29.9	25.0
Copper, Cu (µg/g)	2.8	1.9	6.6
Iron, Fe (µg/g)	4512.9	1091.6	1622.5
Manganese, Mn (µg/g)	54.6	19.2	37.2
Lead, Pb (µg/g)	10.5	6.5	12.6
Zinc, Zn (µg/g)	44.5	28.3	30.6
Sediment at Point 4 – S4			
Cadmium, Cd (µg/g)	0.0	0.0	0.0
Chromium, Cr (µg/g)	130.0	18.6	20.1
Copper, Cu (µg/g)	130.2	8.4	29.1
Iron, Fe (µg/g)	3605.9	1146.0	1664.0
Manganese, Mn (µg/g)	32.4	17.8	26.0
Lead, Pb (µg/g)	0.9	0.2	1.2
Zinc, Zn (µg/g)	34.3	23.5	30.6

Heavy Metals concentration in seagrass samples in September, October, and November 2016

Parameters (mg/L)	Sampling Batch		
	Sep	Oct	Nov
Seagrass at Point 3 – G3			
Cadmium, Cd (µg/g)	0.0	0.0	0.0
Chromium, Cr (µg/g)	143.5	31.0	52.9
Copper, Cu (µg/g)	23.2	0.0	17.5
Iron, Fe (µg/g)	3084.4	608.5	4951.7
Manganese, Mn (µg/g)	20.6	9.3	41.9
Lead, Pb (µg/g)	5.7	9.9	0.1
Zinc, Zn (µg/g)	89.0	23.4	41.5
Seagrass at Point 4 – G4			
Cadmium, Cd (µg/g)	0.0	0.0	0.0
Chromium, Cr (µg/g)	151.5	9.1	20.0
Copper, Cu (µg/g)	11.6	0.0	0.2
Iron, Fe (µg/g)	2667.2	786.0	783.3
Manganese, Mn (µg/g)	103.6	93.0	96.3
Lead, Pb (µg/g)	2.4	3.5	2.3
Zinc, Zn (µg/g)	50.9	21.6	32.9

Comparison of heavy metals absorption in seagrass with sediment in September, October, and November 2016

Month	Sampling Point	Heavy Metal	Sediment	Seagrass	Difference (%)
September	3 Sp. Enhalus Acoroides	Cd	0.0	0.0	0
		Cr	215.5	143.5	-50
		Cu	2.8	23.2	88
		Fe	4512.9	3084.4	-46
		Mn	54.6	20.6	-165
		Pb	10.5	5.7	-84
		Zn	44.5	89.0	50
	4 Sp. Halophilia Ovalis	Cd	0.0	0.0	0
		Cr	130.0	151.5	14
		Cu	0.0	11.6	100
		Fe	3605.9	2667.2	-35
		Mn	32.4	103.6	69
		Pb	0.9	2.4	63
		Zn	34.3	50.9	33
October	3 Sp. Enhalus Acoroides	Cd	0.0	0.0	0
		Cr	29.9	31.0	4
		Cu	1.9	0.0	-100
		Fe	1091.6	382.8	-185
		Mn	19.2	9.3	-107
		Pb	6.5	9.9	34
		Zn	28.3	23.4	-21
	4 Sp. Halophilia Ovalis	Cd	0.0	0.0	0
		Cr	18.6	9.1	-104
		Cu	8.4	0.0	-100
		Fe	1146.0	786.0	-46
		Mn	17.8	93.0	81
		Pb	0.2	3.5	93
		Zn	23.5	21.6	-9
November	3 Sp. Enhalus Acoroides	Cd	0.0	0.0	0
		Cr	25.0	52.9	53
		Cu	6.6	17.5	62
		Fe	1622.5	4951.7	67
		Mn	37.2	41.9	11
		Pb	12.6	0.1	-100
		Zn	30.6	41.5	26
	4 Sp. Halophilia Ovalis	Cd	0.0	0.0	0
		Cr	20.1	20.0	0
		Cu	29.1	0.2	-99
		Fe	1664.0	783.3	-112
		Mn	26.0	96.3	73
		Pb	1.2	2.3	48
		Zn	30.6	32.9	7