Macro and micro nutrients of tropical seagrasses, *Halophila ovalis*, *H. spinulosa* and *Halodule uninervis* in Johore, Malaysia

Wan Hazma, W.N.[1]; Muta Harah, Z.[1,2*]; Japar Sidik B.[3]; Natrah F.M.I.[1,2]

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Abstract

The present study determined and compared contents of macro- (Nitrogen-N, Phosphorus-P, Potassium-K, Calcium-Ca, Magnesium-Mg) and micro- (Zinc-Zn, Iron-Fe, Manganese-Mn, Cupper-Cu) nutrients in four seagrass species; big-leaved (BL) *Halophila ovalis* (R. Br.) Hook. *f*., small-leaved (SL) *H. ovalis* (R. Br.) Ascherson, *H. spinulosa* (R. Br.) Aschers. in Neumayer and *Halodule uninervis* (Forssk.) Aschers from three locations; Merambong shoal, Seluyong shoal-1 and Seluyong shoal-2, Johore, Malaysia on March 2013. Dried seagrass samples were subjected to wet digestion method and concentration determined using a Perkin Elmer 5100 PC atomic absorption spectrophotometer. Macro and micro-nutrients varied with species and locations. Observed macro-nutrient contents were high for N (17320 mg kg^{-1}) in big-leaved (BL) *H. ovalis* from Seluyong shoal-2, P (2921.33 mg kg^{-1}) in big-leaved (BL) *H. ovalis* from Seluyong shoal-1, and for K (10942.67 mg kg^{-1}) in *H. uninervis* from Merambong shoal and for Ca (10576 mg kg^{-1}) in small-leaved (SL) *H. ovalis* from Merambong shoal. Mg (3534.67 mg kg^{-1}) was comparatively higher in *H. spinulosa* from Merambong shoal. Micro-nutrients such as Zn ranged from 138.67 mg kg^{-1} to 212 mg kg^{-1} and Cu from 13.33 mg kg^{-1} to17.33 mg kg^{-1} with no discernible differences between species. Fe concentration was consistently higher in all species. Hierarchically macro and micro-nutrients contents did not show a consistent ordered pattern according to species and location. Based on biplots generated by Principle component analysis (PCA), there were clear separation of seagrass species based on the micro- and macro-nutrient contents with locations from Malaysia and other regions.

Keywords: *Halodule* sp., *Halophila* sp., Macro-nutrient, Micro-nutrient, PCA, Malaysia

1-Department of Aquaculture, Faculty of Agriculture, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor Darul Ehsan, Malaysia.

2-Institute of Bioscience, Universiti Putra Malaysia, UPM Serdang, Selangor Darul Ehsan, Malaysia.

3-Faculty of Agriculture and Food Sciences, Universiti Putra Malaysia Bintulu Sarawak Campus, Nyabau Road, 97008 Bintulu, Sarawak, Malaysia.

*Corresponding author's email: muta@upm.edu.my

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Introduction

Seagrasses are marine angiosperms and are known for their important role in ecology closely related to coastal zone economy, due to high primary and secondary productive tropical ecosystem (Hemminga and Duarte, 2000; Bronwyn, 2006). Huge extensive meadows are always vital to near shore fisheries, acting as natural source of food and shelter for juvenile fishes, marine invertebrates, marine reptiles and mammals (Duarte, 2002; Japar Sidik and Muta Harah, 2003). It is well accepted that seagrass affects the chemical, physical and biological environment significantly, and is therefore considered as an "ecological engineer" (McKenzie, 2008). Seagrass habitation depends on suitable substrates to anchor and nutrient rich sediments from river upstream normally deposited within first few kilometers from the river mouth and estuarine (Larcombe and Woolfe, 1999). Seagrasses not only inhabited deposited substrates, but also play an important role in trapping sediments and stabilize them with vast root mat and at the same time absorb nutrients to flourish both seagrass beds and their interrelated environment (Fonseca, 1989; Gacia *et al*., 2003). According to Malea (1994), seagrasses uptake higher concentrations of potassium, magnesium, calcium, iron and sodium which take part in the physiological processes of aquatic plants that are influenced by the abundance of the nutrient concentrations in the sediments and dissolved nutrients in the seawater. Although seagrasses are widespread both in temperate and tropical areas, they do not represent similarity with respect to their adaptation to the chemical, physical and biological environment. They may have different optimal requirements based on climate regimes. Chemical analyses have been carried out on several seagrass species partly to obtain information linked to nutrient resources (Short, 1987), organisms and detrital food chains, nutritional status and potential food source for human utilization. According to Kannan *et al*. (1992), seagrass, *H. ovalis* can be used as medicine to treat various skin diseases, burns and boils among Tamilnadu, South India fishing communities. A unique marine based product can give an useful effect to the body, for example, anti-HIV agents isolated from *Thalassia testudinum* was beneficial from a medical prospective (Rowley *et al*., 2002). Studies of the variation of multi-nutrient especially trace elements in the tropical seagrasses have been carried out by Nienhuis (1986), in the Europe by Malea *et al*. (1994), Malea (1994), Malea and Haritonidis (1995), Kannan *et al*. (2011), Kilminster (2013) and Thangaradjou *et al.* (2013). Although there has been much focus on heavy and trace metals of the seagrasses in the world over the last three decades, studies on their nutritional values and their possible utilization as the substitutes for the existing food sources are still lacking. In the present study macro-nutrients (N, P, K, Ca and Mg) and micro-nutrients (Zn, Fe, Mn and Ca) in tropical seagrasses; *H. ovalis*, *H. spinulosa* and *H. uninervis* from Seluyong shoal-1, Seluyong shoal-2 and Merambong shoal, Johore, Malaysia were determined and compared with other available nutrient data from temperate area, Antikyra Gulf, Greece and several areas from India. The significance of this present study of nutrient contents possesses immense value to evaluate seagrass for use as food for human consumption and industrial uses.

Materials and methods

Study area and sample collection

Field studies were conducted on March 2013 at Merambong shoal (1º19'59.86″N, 103º39'0.13″E) and Seluyong shoal-1 (1º22'03.26"N, 103º38' 03.23"E) and Seluyong shoal-2 (1º21'10.59″N, 103º37'05.73″E), Johore, Malaysia. Figure 1a shows the location of Merambong shoal, Seluyong shoal-1 and Seluyong shoal-2 Johore, Malaysia. Four seagrass species; big-leaved (BL) *H. ovalis* (Seluyong shoal-1, Seluyong shoal-2 and Merambong shoal), small-leaved (SL) *H. ovalis* (Merambong shoal); *H. spinulosa* (Seluyong shoal-2 and Merambong shoal) and *H. uninervis* (Merambong shoal) were randomly collected during the low tide (Fig. 1b). Samples were washed thoroughly in seawater to remove all sediment then placed in sealed polythene plastic bags, preserved on ice in an ice chest and transported to the laboratory prior to sorting for analysis. In the laboratory, samples were rinsed with distilled water to remove dirt and epiphytes. Samples were dried at 60°C for a week to constant weight (Nienhuis, 1986), ground with a grinder (Panasonic MX-337) to a powder and kept in air tight plastic bottles, placed in a desiccator at room temperature for nutrient analysis.

Nutrients analysis of seagrasses

0.25 g dry weight of sample was added to 5 ml concentrated sulphuric acid (H_2SO_4) and 2 ml 50% hydrogen peroxide (H_2O_2) and heat digested with a stepwise increase of temperature to 285°C. The digested sample was diluted with distilled water and analyzed for macro-nutrients (N, P, K, Ca and Mg) and micro-nutrients (Fe, Zn, Mn and Cu) using a Perkin Elmer 5100 PC atomic absorption spectrophotometer equipped with a single hollow cathode lamp for each element and an air-acetylene burner, against mineral elements standards. The determination of macro-and micro-nutrients in seagrasses for the three locations; Seluyong shoal-1, Seluyong shoal-2 and Merambong shoal was done in triplicate (AOAC, 1990).

Statistical analysis

Macro- and micro-nutrient concentrations of seagrass species were compared using one way analysis of variance (ANOVA) and Duncan's New Multiple Range Test (DNMRT, $p<0.05$ (Zar, 1999) following IBM SPSS Statistics 21 for Windows software. PCA based on Bray Curtis similarity index was carried out using XLSTAT software (Window version 2013) to obtain the relationship between Macro- and micro-nutrients and, seagrass species in this study and available data from other regions. PCA was the most common multivariate statistical methods used in environmental studies (Thangaradjou *et al*., 2013).

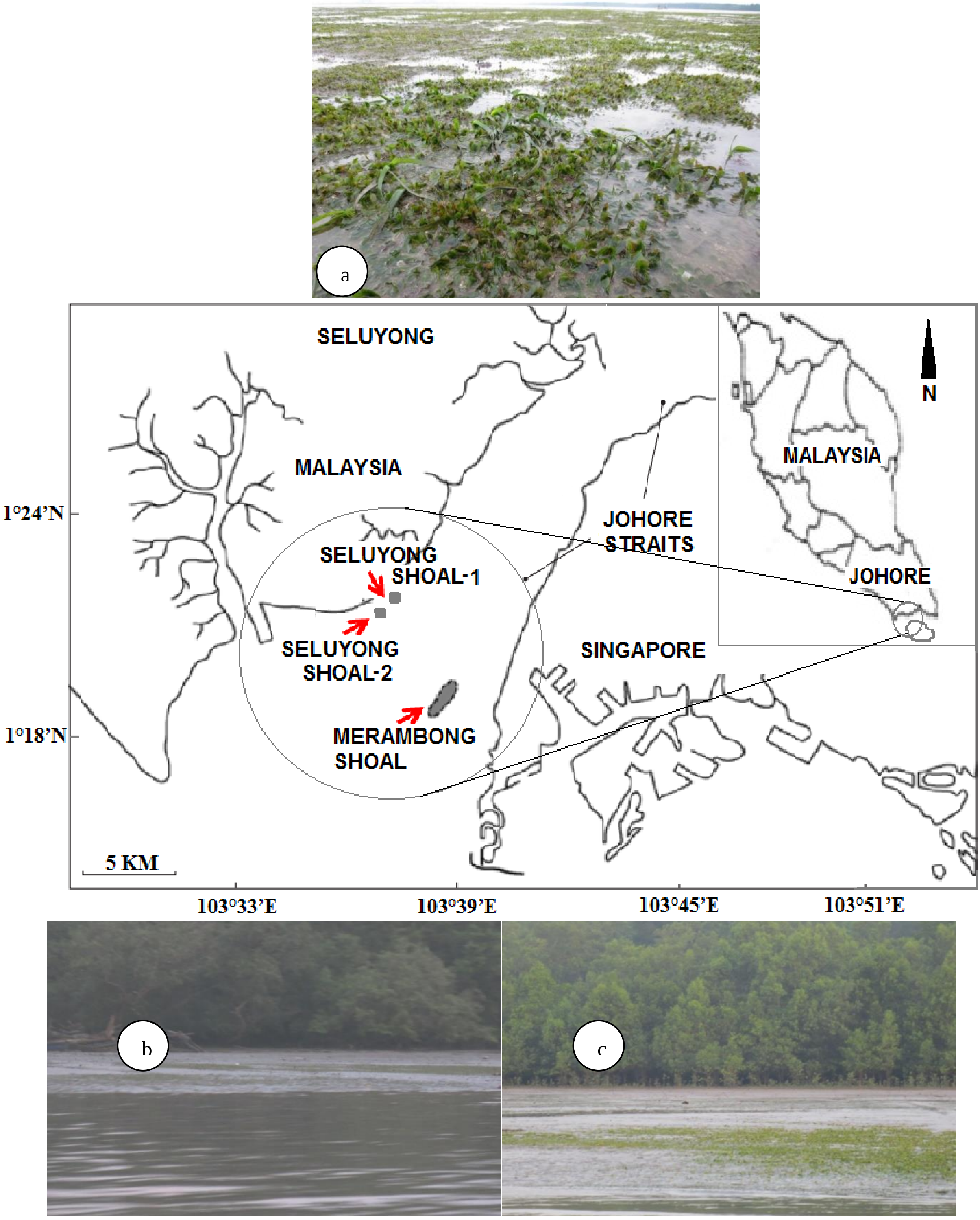

Figure 1a: Location of sampling study of Johore Strait (Tebrau), Johore, Malaysia a) Merambong shoal b) Seluyong shoal-1 and c) Seluyong shoal-2.

Figure 1b: Seagrass species that was randomly collected from Seluyong shoal-1, Seluyong shoal-2 and Merambong shoal a) *Halophila spinulosa* b) *Halodule uninervis* c) small-leaved *H. ovalis* d) big-leaved *Halophila ovalis*.

Results

Macro-nutrient of seagrasses

Macro-nutrient concentrations and their trend are summarized in Table 1. The three species of seagrass were ordinated with PCA using macro-nutrient as variables (N, P, K, Ca and Mg). In a principal component analysis the first two principal components, PC1 and PC2 accounted for 43.0% and 28.0% respectively of the total variations (Table 2). The eigenvectors of the 5 variables are presented in Table 3. In PC1 except for Ca with negative relationship, there were positive relationships for N, P, K and Mg. However, K was positively correlated with PC2 while the rest showed a negative relationship. Based on the Bray-Curtis Similarity index at 50% similarity, seagrass species were clustered into 4 main Groups (Tables 1, 2, 3 and 4, Fig. 2). Group 1 comprised *H. spinulosa* b, BL *H. ovalis* a and BL *H. ovalis* b with higher N, P and Mg content. Group 2 *H. spinulosa* c and BL *H. ovalis* c with moderate P, N and K content. Group 3 consisted of SL *H. ovalis* c only with high Ca content while Group 4 *H. uninervis* c had high K content. The findings also indicated that *H. ovalis* and *H. spinulosa* were clustered in separate group according to location.

High N content was observed in BL *H. ovalis* b, 17320 mg kg^{-1} and high P content in BL *H. ovalis* a with 3060 mg kg^{-1}. SL *H. ovalis* c had a higher value of Ca (10576 mg kg^{-1}) while BL *H. ovalis* a possessed a lower value (4705.33 mg kg^{-1}). Potassium

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(K) content in *Halodule uninervis* c was comparatively higher with a value of 10942.67 mg kg^{-1}. Magnesium (Mg) content was highest in *H. spinulosa* c followed by *H. spinulosa* b and BL *H. ovalis* c with 3534.67 mg kg^{-1} and 3510.67 mg kg^{-1}, respectively.

Analysis in trends for macro-nutrient (N, P, K, Ca, Mg) content revealed seagrass varied in content values with respect to species and location (Table 1). BL *H. ovalis* c, BL *H. ovalis* a, BL *H. ovalis* b and *H. spinulosa* b, *H. spinulosa* c and *H. uninervis* c accumulated higher N and SL *H. ovalis* c higher Ca. Phosphorus (P) content was the lowest followed by Mg in all species.

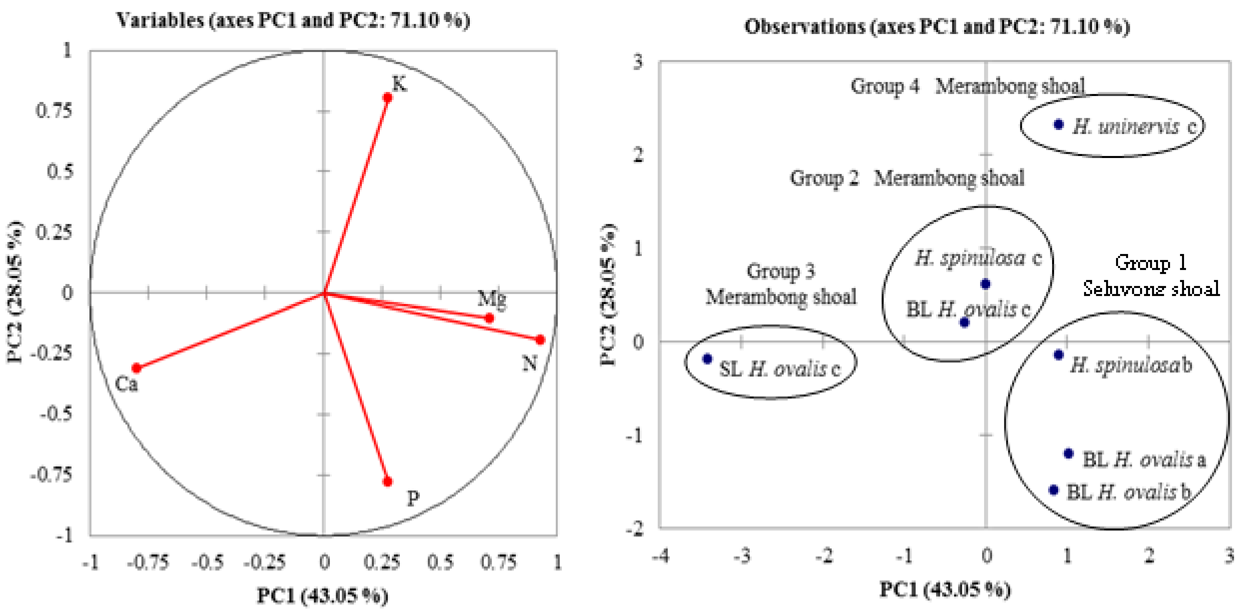

Figure 2: The distribution of three seagrass species (with their locations) against the first two PC's scores. Group 1, 2, 3 and 4 are based on Bray Curtis similarity index. a=Seluyong shoal-1; b=Seluyong shoal-2 and c=Merambong shoal.

Table 1: Macro-nutrient of seagrasses in three different locations.

Species	**Macro nutrients (mg kg^{-1} DW)**					**Trend in nutrient content**
	N	**P**	**K**	**Ca**	**Mg**	
BL *H. ovalis* a	15093.33±127.19^{b}	3060.00±23.09^{a}	7148.00±51.59^{b}	4705.33±414.31^{c}	3404.00±6.11^{bc}	N>K>Ca>Mg>P
BL *H. ovalis* b	17320.00±260.26^{a}	2921.33±36.10^{b}	4126.67±28.88^{e}	8009.33±137.49^{b}	3430.67±10.41^{b}	N>Ca>K>Mg>P
H. spinulosa b	14026.67±70.55^{c}	1536.00±10.58^{f}	7152.00±32.33^{b}	5361.33±158.50^{c}	3510.67±9.33^{a}	N>K>Ca>Mg>P
BL *H. ovalis* c	13000.00±415.69^{d}	2058.67±46.78^{c}	5590.67±50.82^{c}	8204.00±205.56^{b}	3510.67±3.53^{a}	N>Ca>K>Mg>P
SL *H. ovalis* c	8106.67±104.14^{e}	2072.00±18.90^{c}	4814.67±68.52^{d}	10576.00±212.80^{a}	3244.00±12.22^{d}	Ca>N>K>Mg>P
H. uninervis c	14800.00±151.44^{b}	1745.33±17.64^{d}	10942.67±121.60^{a}	4814.67±70.67^{c}	3381.33±10.91^{c}	N>K>Ca>Mg>P
H. spinulosa c	13413.33±246.94^{cd}	1637.33±26.77^{e}	5440.00±81.22^{c}	8241.33±54.57^{b}	3534.67±13.33^{a}	N>Ca>K>Mg>P

Data are mean values ± S.E in column with different superscript alphabet (a>b>c>d>e>f) are significantly different (p<0.05) DMRT, N=3, DW=dry weight; a=Seluyong shoal-1; b=Seluyong shoal-2 and c=Merambong shoal

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Table 2: Principle component analysis (PCA) for macro-nutrient: Eigenvalues and percentage of variation described by the first five components.

PC	1	2	3	4	5
Eigenvalue	2.2	1.4	0.9	0.4	0.1
Variability (%)	43.0	28.0	18.7	8.3	1.9
Cumulative %	43.0	71.1	89.8	98.1	100.0

Table 3: Eigenvectors for variables, the macro-nutrient used in PCA, indicating the strength of the correlation between variables and the principle components (axes).

PC	1	2	3	4	5
N	0.927	-0.193	-0.122	0.213	0.207
P	0.278	-0.782	-0.539	0.028	-0.144
K	0.276	0.805	-0.425	0.293	-0.099
Ca	-0.797	-0.310	0.112	0.505	0.026
Mg	0.710	-0.103	0.661	0.165	-0.147

Table 4: Micro-nutrient of seagrasses in three different locations.

Species	Micro nutrients (mg kg^{-1} DW)				Trend in nutrient content
	Zn	Fe	Mn	Cu	
BL *H. ovalis* a	146.67±21.21^{b}	3793.33±640.88^{b}	173.33±2.67^{a}	16.00±0.00^{bc}	Fe>Mn>Zn>Cu
BL *H. ovalis* b	156.00±10.07^{b}	5954.67±186.71^{a}	149.33±1.33^{b}	13.33±2.67^{c}	Fe>Zn>Mn>Cu
H. spinulosa b	212.00±40.60^{a}	5782.67±236.02^{a}	154.67±1.33^{b}	17.33±2.67^{a}	Fe>Zn>Mn>Cu
BL *H. ovalis* c	138.67±5.81^{b}	5142.67±910.12^{ab}	122.67±3.53^{c}	13.33±1.33^{c}	Fe>Zn>Mn>Cu
SL *H. ovalis* c	152.00±8.33^{b}	3737.33±25.44^{cd}	113.33±1.33^{d}	16.00±0.00^{bc}	Fe>Zn>Mn>Cu
H. uninervis c	162.67±3.53^{b}	4516.00±158.05^{bc}	60.00±0.00^{f}	13.33±2.67^{c}	Fe>Zn>Mn>Cu
H. spinulosa c	156.00±6.11^{b}	5146.67±355.28^{ab}	93.33±1.33^{e}	17.33±1.33^{a}	Fe>Zn>Mn>Cu

Data are mean values ±S.E in column with different superscript alphabet (a>b>c>d>e>f) are significantly different (p<0.05) DMRT, N=3, DW=dry weight; a=Seluyong shoal-1; b=Seluyong shoal-2 and c=Merambong shoal

Micro-nutrient of seagrasses

Micro-nutrient concentrations and their trends are presented in Table 4. The three species of seagrass were ordinated with PCA using micro-nutrient (Fe, Zn, Mn and Cu) concentration as variables. The first two PCs accounted for 71.62% of total variance for micro-nutrient and species studied (Table 5 and Fig. 3). PC1 explained the high percentage of variance (43.43%) compared to PC2 (28.19%). The biplot generated four main groups as in micro-nutrient analysis and the grouping of species were distinctly different. All the variables (micro-nutrients) were loaded on the positive sites of PC1 (Table 6 and Fig. 3). Group 1 was composed of species; BL *H. ovalis* b and *H. spinulosa* b and possessed higher Fe and Zn content. The Group 2 consisted of *H. spinulosa* c with

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high Cu and moderate Mn content. SL *H. ovalis* c and BL *H. ovalis* a were in Group 3 with moderate micro-nutrient content. Group 4 consisted of BL *H. ovalis* c and *H. uninervis* c with moderate Fe and Zn and lower Cu and Mn content. There was no significant difference between the species for zinc and copper.

Table 5: Principle component analysis (PCA) for micro-nutrient: Eigenvalues and percentage of variation described by the first four components.

PC	1	2	3	4
Eigenvalue	1.737	1.128	0.863	0.272
Variability (%)	43.431	28.193	21.564	6.812
Cumulative %	43.431	71.624	93.188	100.000

Table 6: Eigenvectors for variables, the micro-nutrient used in PCA, indicating the strengths of the correlations between variables and the principle components (axes).

PC	1	2	3	4
Zn	0.867	0.147	-0.336	0.337
Fe	0.541	0.790	0.104	-0.269
Cu	0.515	-0.262	0.813	0.075
Mn	0.653	-0.643	-0.281	-0.284

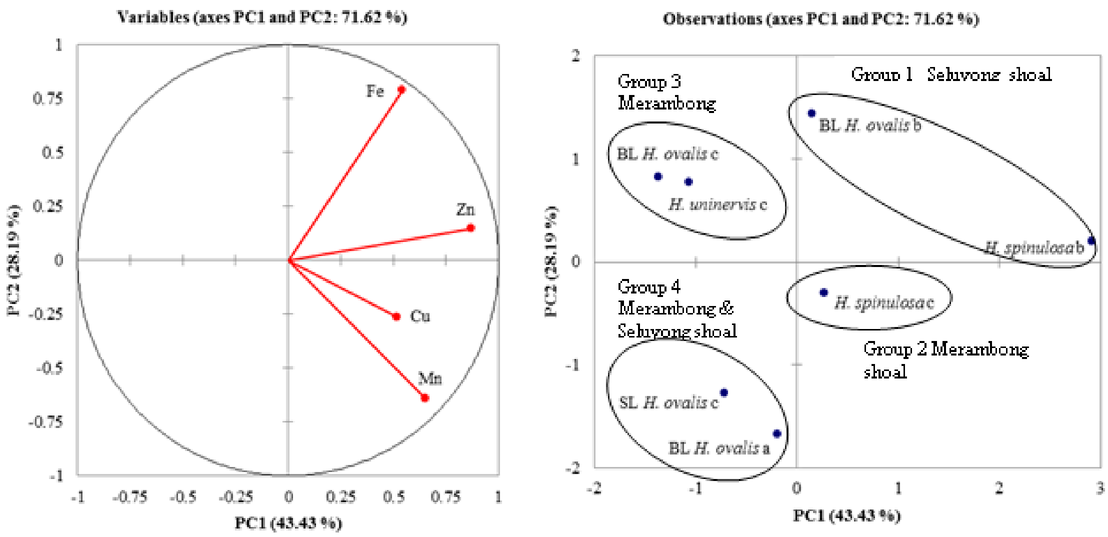

Figure 3: The distribution of three seagrass species (with their locations) against the first two PC's scores. Group 1, 2, 3 and 4 are based on Bray Curtis similarity index. a=Seluyong shoal-1; b=Seluyong shoal-2 and c=Merambong shoal.

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Discussion

Macro-nutrient of seagrasses

Macro-nutrient such as N, P and K are essential elements (Preen, 1995) and seagrass themselves require these elements for growth and perpetuation of other interrelated populations (seaweeds and other organisms) in the environment (Fonseca, 1989; Gacia *et al*., 2003). Many marine and estuarine organisms utilize seagrasses for food. Seagrasses; *Enhalus acoroides*, *H. ovalis*, *H. spinulosa*, *Cymodocea serrulata*, *H. pinifolia*, *H. uninervis* and *Syringodium isoetifolium* (Lanyon *et al*., 1989; Supanwanid, 1996) are food for large species, such as *Dugong dugon* (dugong, status: vulnerable, VU A1cd) and *Chelonia mydas* (green turtle, status: endangered, EN A1bd). Both species are present in the seagrass areas under study (Japar Sidik and Muta Harah, 2003). In contrast, the findings by Yamamuro and Chirapart (2005) for seagrasses collected along the coast of Thailand indicated that *H. ovalis* contained less N and more ash compared to other seagrasses such as *E. acoroides*, *T. hemprichii* and *C. rotundata*. Calcium and magnesium content were higher in SL *H. ovalis* c and *H. spinulosa* c, which were 10576 mg kg^{-1} and 3534.67 mg kg^{-1} respectively compared to other seagrasses. At our study sites, the seagrass species contained higher Ca compared to seaweed species such as *Gracilaria changii* at 6510 mg kg^{-1} (Norziah and Ching, 2000). According to Heaney (1996) in human, calcium is important for bones, and without an adequate intake, it is not possible either to build or maintain a fully normal skeletal mass.

Micro-nutrient of seagrasses

Seagrasses from Seluyong shoal-2 have high Fe content. Big-leaved (BL) *H. ovalis* from Seluyong shoal-1 have the highest Mn content at 173.33 mg kg^{-1}. Zinc (Zn), Fe, Cu and Mn are essential micro-nutrients for plants and can be toxic at higher concentrations than normal amounts requirement for growth (Kannan *et al*., 2011). In the human body iron is important in hemoglobin formation and transfer of oxygen and electrons (Bhowmik *et al*., 2012). According to MacArtain *et al*. (2007), trace elements such as zinc have negative health effects if it is consumed in high quantities. Marine seaweed such as *G. changii* have high concentrations of elements relative to vegetables such as green beans, tomato, broccoli and red chilli, as its metabolic system can absorb elements directly from the seawater (Norziah and Ching, 2000). The trend of micro-nutrient content showed Fe was highest and Cu was the lowest in all species. Similar trends in content were also reported for other seagrasses by Denton *et al*. (1980).

Comparison of present study with other available data

Macro-and micro-nutrient content data in seagrass species of this study were compared with available data from other regions. Eleven (11) seagrass species, with different locations were ordinated with PCA (Table 7 and Fig. 4) using 3 variables for macro-nutrients (K, Ca and Mg) and 4 variables for micro-nutrients (Zn, Fe, Cu and Mn). Based on the macro-nutrient variables, the first two factors explain 97.75% of total variance. PC1 explained higher variance of 91.70% of the total

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variance (Table 8) and was strongly influenced by K, Mg and Ca while PC2 was not dominated by any variables with 6.05% of total variance. The biplot categorized the seagrass species and their macro-nutrient into three groups (Fig. 5a). Group 1 are seagrasses (from Greece, *H. stipulacea* GR5) and *H. ovalis* MYb, *H. ovalis* MYc and *H. spinulosa* MYc with high content of magnesium and calcium. Group 2 consisted of multiple seagrass species, BL *H. ovalis* MYa, *H. spinulosa* MYb and *H. uninervis* MYc (from Malaysia) and *Posidonia oceanica* GR5 from Greece. These species have significantly higher K compared to other seagrasses from various locations. Group 3 comprised seagrass, *E. acoroides* IN2 (*Enhalus acoroides*), *T. hemprichii* IN2 (*Thalassia hemprichii*), *C. rotundata* IN2 (*Cymodocea rotundata*), *C. serrulata* IN2 (*Cymodocea serrulata*), *H. pinifolia* IN2 (*Halodule pinifolia*) and *S. isoetifolium* IN2 (*Syringodium isoetifolium*) from Gulf of Mannar, India with lower macro-nutrient values compared to other species.

With respect to micro-nutrient variables ordinated with PCA, PC1 (57.51%) and PC2 (28.78%) explained 86.29% of total variance (Table 9). The PCA resulted in four major groups (Fig. 5b). All seagrasses from Malaysia were grouped together in Group 1 with higher Zn and Fe content. Group 2 are seagrass species, *H. decipiens* IN3 (from Lakshadweep island, India) and, *H. ovalis* IN1, *H. ovata* IN1 (*Halophila ovata*), *C. rotundata* IN1, *H. uninervis* IN1 (*Halodule uninervis*), *H. pinifolia* IN1 (*Halodule pinifolia*) and *S. isoetifolium* IN1 from Andaman Island, India. These species have high Mn content. Group 3 are seagrasses, *E. acoroides* IN2, *C. rotundata* IN2 and IN3, *C. serrulata* IN2 and IN3, *H. uninervis* IN3, *H. pinifolia* IN2 and IN3, and *S. isoetifolium* IN2 and IN3 from Gulf of Mannar and Lakshadweep Island, India with lower micro-nutrient content. *Halodule pinifolia* IN4 and *E. acoroides* IN1 from Palk Bay and Andaman Island respectively were clustered in Group 4 with lower micro-nutrient content. The comparative analysis suggested that there is a distinct separation of seagrass species in terms of macro- and micro-nutrient with location. Many factors e.g., chemical, physical and biological environment could influence the nutrient content in seagrass species.

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Table 7: Comparison of macro- and micro-nutrients in seagrass species at different locations (mg kg^{-1}).

Species	Location	Macro-nutrient			Micro-nutrient				Reference
		K	Ca	Mg	Zn	Fe	Cu	Mn	
H. ovalis IN1	Andaman Island				56.68	989.89	91.94	1118	Thangaradjou *et al*. (2010)
H. ovalis MYa	Seluyong shoal-1	7148	4705.33	3404	146.67	3793.33	173.33	16	Present study
H. ovalis MYb	Seluyong shoal- 2	4126.67	8009.33	3430.67	156	5954.67	149.33	13.33	Present study
H. ovalis MYc	Merambong shoal	5590.67	8204	3510.67	138.67	5142.67	122.67	13.33	Present study
H. ovalis MYc	Merambong shoal	4814.67	10576	3244	152	3737.33	113.33	16	Present study
H. spinulosa MYb	Seluyong shoal-2	7152	5361.33	3510.67	212	5782.67	154.67	17.33	Present study
H. spinulosa MYc	Merambong shoal	5440	8241.33	3534.67	156	5146.67	93.33	17.33	Present study
E. acoroides IN1	Andaman Island				28.88	525.6	30.52	508	Thangaradjou *et al*. (2010)
E. acoroides IN2	Gulf of Mannar	240.1	220.233	732.8	5.24	90.45	3.59	7.17	Kannan *et al*. (2011)
C. serrulata IN2	Gulf of Mannar	10	19.4	905.5	13.57	69.4	7.49	12.83	Kannan *et al*. (2011)
C. serrulata IN3	Lakshadweep Island				55.52	175.5	7.91	17.56	Thangaradjou *et al*. (2013)
C. rotundata IN1	Andaman Island				72.14	1132.8	100.3	1725	Thangaradjou *et al*. (2010)
C. rotundata IN2	Gulf of Mannar	17.3	25.5	91.4	13.95	71.2	7.8	13.46	Kannan *et al*. (2011)
C. rotundata IN3	Lakshadweep Island				51.69	124.3	4.87	19.97	Thangaradjou *et al*. (2013)
H. uninervis IN1	Andaman Island				80.24	994.2	78.52	1902	Thangaradjou *et al*. (2010)
H. uninervis IN3	Lakshadweep Island				30.69	5737.1	17.5	129.1	Thangaradjou *et al*. (2013)
H. uninervis MYc	Merambong shoal	10942.67	4814.67	3381.33	162.67	4516	60	13.22	Present study
H. pinifolia IN4	Palk Bay				69.17	1886.6	60.8	491.6	Kannan *et al*. (1992)
H. pinifolia IN1	Andaman Island				64.05	809.05	98.92	1300	Thangaradjou *et al*. (2010)
H. pinifolia IN2	Gulf of Mannar	28.116	25.07	863.4	17.59	156.5	6.65	23.42	Kannan *et al*. (2011)
H. pinifolia IN3	Lakshadweep Island				42.54	5992.2	10.7	101.2	Thangaradjou *et al*. (2013)
H. decipiens IN3	Lakshadweep Island				60.96	5982.6	21.5	1255	Thangaradjou *et al*. (2013)
H. ovata IN1	Andaman Island				51.5	965.08	97.38	1281	Thangaradjou *et al*. (2010)
S. isoetifolium IN1	Andaman Island				85.5	1920.4	109.5	2224	Thangaradjou *et al*. (2010a)
S. isoetifolium IN2	Gulf of Mannar	300.9	181.15	912.7	6.54	82.01	3.18	14.93	Kannan *et al*. (2011)
S. isoetifolium IN3	Lakshadweep Island				27.36	155.1	6.04	25.78	Thangaradjou *et al*. (2013)
T. hemprichii IN2	Gulf of Mannar	270.33	100.166	591.1					Kannan *et al*. (2011)
P. oceanica GR5	Antikyra Gulf, Greece	24320	13285	6791					Malea *et al*. (1994)
H. stipulacea GR5	Antikyra Gulf, Greece	19816	19657	13497					Malea (1994)

Country code: MY=Malaysia; IN=India; GR=Greece. Location: 1=Andaman Island; 2=Gulf of Mannar; 3=Lakshadweep Island; 4=Palk Bay; 5=Antikyra Gulf; a=Seluyong shoal-1; b=Seluyong shoal-2 and c=Merambong shoal

Table 8: Principle component analysis (PCA) for Macro-nutrient (K, Ca and Mg). Eigenvalues and percentage of variation described by the first three components.

PC	1	2	3
Eigenvalue	2.751	0.181	0.067
Variability (%)	91.702	6.050	2.249
Cumulative %	91.702	97.751	100.000

Table 9: Principle component analysis (PCA) for Micro-nutrient (Zn, Fe, Mn and Cu). Eigenvalues and percentage of variation described by the first four components.

PC	1	2	3	4
Eigenvalue	2.300	1.151	0.447	0.101
Variability (%)	57.505	28.782	11.177	2.536
Cumulative %	57.505	86.287	97.464	100.000

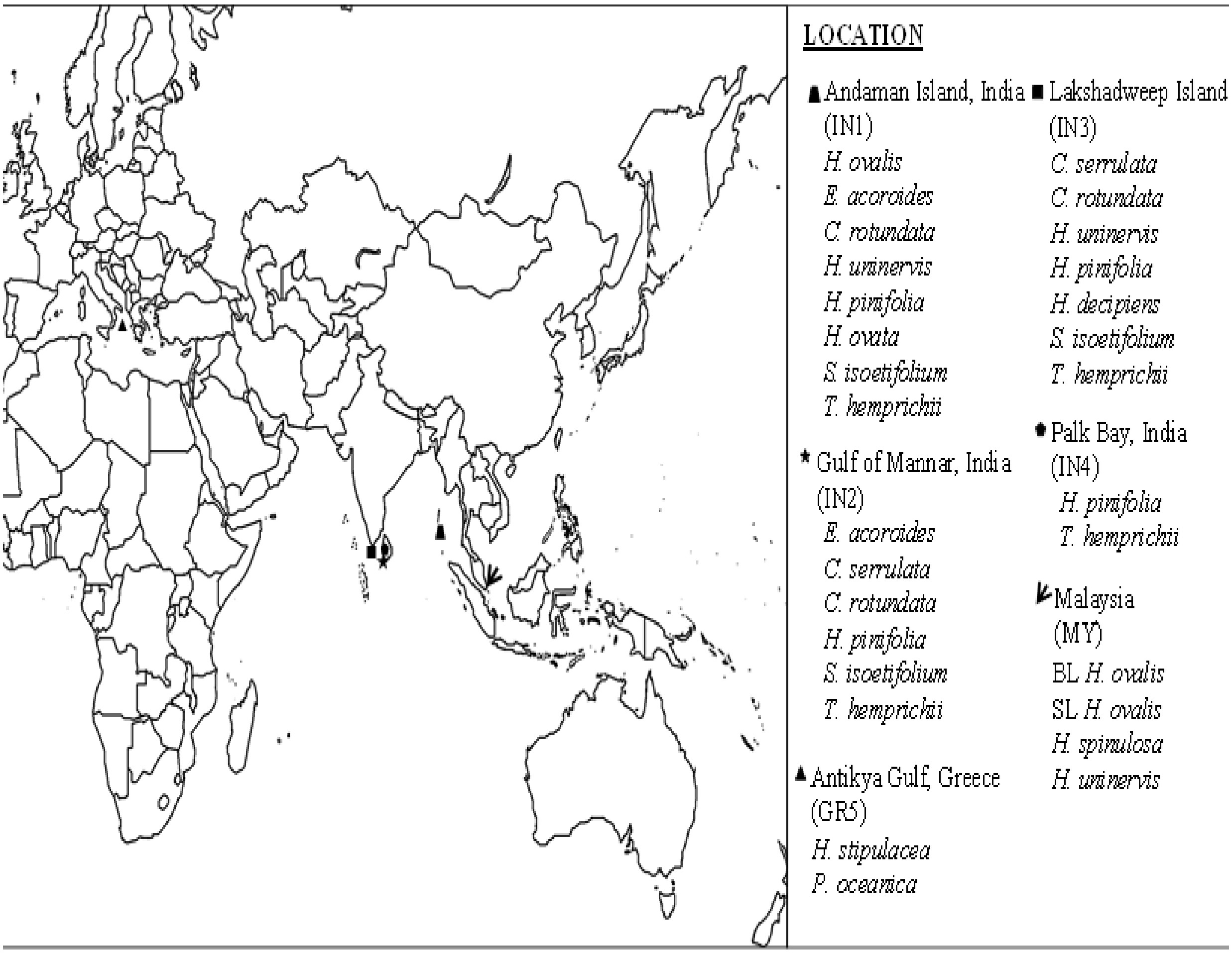

Figure 4: Distribution of seagrass species and their macro- and micro-nutrient in various and this present study. Country code: MY=Malaysia; IN=India; GR=Greece. Location: 1=Andaman Island; 2=Gulf of Mannar; 3=Lakshadweep Island; 4=Palk Bay; 5=Antikyra Gulf.

[DOR: 20.1001.1.15622916.2015.14.1.19.6]

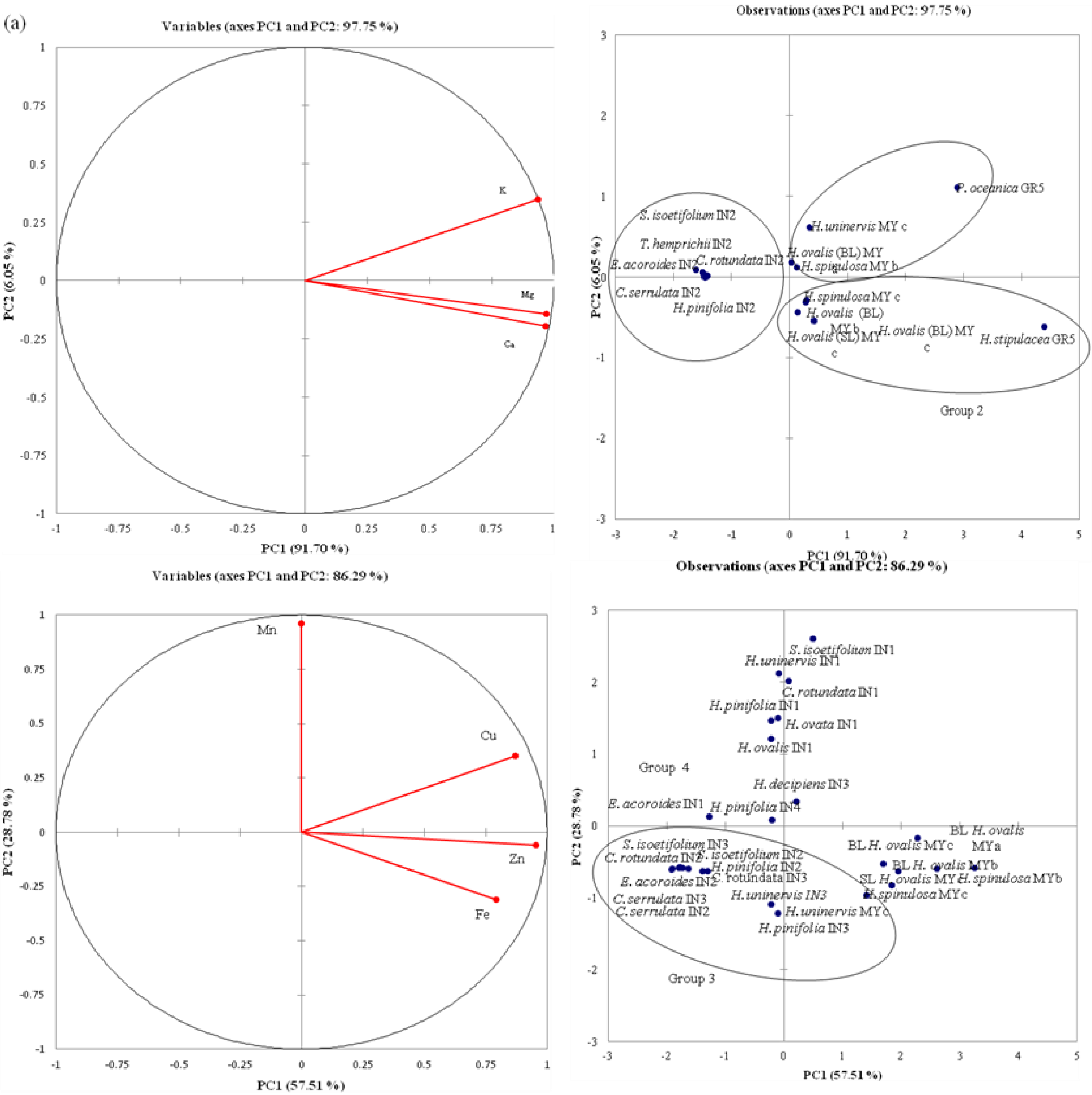

Figure 5: The distribution of three seagrass species (with their locations) against the first two PC's scores. Group 1, 2, 3 and 4 are based on Bray Curtis similarity index. (a) macro-nutrient and (b) micro-nutrient of seagrasses in comparison with other available data. Country code: MY=Malaysia; IN=India; GR=Greece. Location: 1=Andaman Island; 2=Gulf of Mannar; 3=Lakshadweep Island; 4=Palk Bay; 5=Antikyra Gulf; a=Seluyong shoal-1; b=Seluyong shoal-2 and c=Merambong shoal.

From this study it could be concluded that BL *H. ovalis* b (from Seluyong shoal-2) have the highest nitrogen content, 17320 mg kg^{-1} followed by calcium (10576 mg kg^{-1}) in SL *H. ovalis* c (from Merambong shoal) and potassium (10942.67 mg kg^{-1}) in *H. uninervis* (from Merambong shoal). Macro- and micro-nutrient content varied with species and location. Comparatively seagrass species in relation to nutrient content are clustered in separate groups of the country. Seagrasses of Merambong shoal, Seluyong shoal-1 and Seluyong shoal-2 of Sungai Pulai, Johore are rich in macro- and micro-nutrients and may be linked to nutrient resources, organisms and detrital food chains, nutritional status and potential food sources for human utilization.

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