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SEASONAL VARIATION AND NUTRIENT LEVELS INFLUENCE PHENOLIC CONTENTS IN SEAGRASS *THALASSIA HEMPRICHII* ALONG DAR ES SALAAM COAST

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ABSTRACT

Seasonal variation in total phenolics in Thalassia hemprichii from four different sites with varied nutrient loading along Dar es Salaam coast was studied. Besides few studies existing on phenolics in seagrass species done to assess status of the seagrass meadows, there is paucity of information for seagrass species along the coast of Indian Ocean in Dar es Salaam. In this study, Thalassia hemprichii shoots were collected from four different sites having different levels of anthropogenic pressure during dry and rainy seasons. The total phenol contents of above and belowground parts of the seagrass were determined using Folin-Ciocalteu method. Results indicates significant differences in total phenol from different sites in both above and below ground tissues, ranging from 2.7 to 35.7 µgGAE/mg and 1.9 to 142.9 µgGAE/mg, respectively. The highest concentration of total phenol during dry season was recorded in samples collected from Kunduchi site and the lowest being at Oysterbay site. Similarly, during rainy season significant variation in concentration of total phenols were observed among sites ranging from 2.3 to 26.4 and 12.7 to 77.0 µgGAE/mg for above and belowground parts, respectively. Generally in all sites, a significant higher concentration of total phenol in the belowground parts was observed than in the above ground parts in both seasons and was higher in dry than in rainy season. There was negative correlation between concentration of phenol in the tissues of seagrass and the concentration of nutrients in water. Our finding shows that variation in seasons and nutrient levels influence the accumulation of phenolic compounds in the studied seagrass. Thus phenol content could be used as early warning biological marker for assessing the status of seagrass meadows subjected to multiple environmental pressures.

Key words: Phenolic content, *Thalassia hemprichii*, Seagrass, Nutrient levels, Dar es Salaam coast.

INTRODUCTION

Seagrasses are marine angiosperms that support array of organisms. They play a major role as food source for aquatic herbivores (Heck et al. 2003), provide habitat for many organisms including important commercial fish species, stabilize sediments and minimize ocean erosion. Seagrasses are also very important in maintaining coastal water quality and stimulating biogeochemical cycling (Heiss et al. 2000). They serve as substrata for varied epiphytic community comprising of but not limited to bacteria, microalgae, and macroalgae (Uku 2005, Borowitzka et al. 2006). To human population along the coast, seagrass meadow serves as the fishing ground for commercial and domestic consumption, and ultimately improved living standard of these communities (Gullström et al. 2002, de la Torre-Castro and Rönnbäck 2004). Regardless of invaluable ecosystem services rendered by seagrasses, many studies have reported their deterioration at a global scale mainly due to anthropogenic causes (Short and Wyllie-Echeverria 1996, Duarte 2002, Orth et al. 2006, Waycott et al. 2009). These include but not limited to runoff from domestic sewage, industrial activities and use of fertilizers in agricultural activities. All these add pollutants in the coastal environments and hence causing stress to marine biota (Short and Wyllie-Echeverria 1996, Duarte 2002, Orth et al. 2006, Waycott et al. 2009).

Growing fixed in the sediments, seagrasses encounter many changes that occur in the ocean. For that reason they have been used as the indicator of prevailing conditions in the marine ecosystems. However, the assessment of their ecophysiological characteristics and detection of their decline rely mostly on quantification of their densities, biomass and growth rates; the variables that respond slowly to environmental changes (Pergent-Martini et al. 2005). Under such circumstance, it is usually too late to propose or undertake any remedial measure when devastation has already taken place. Thus the use of biological markers that respond to the environmental changes in short term to detect the inception stress thresholds is imperative for effective conservation of this valuable ecosystem. Total phenolic content is one of the biological markers that could be used to detect and quantify early alterations in the seagrass physiological response (Ferrat et al. 2003).

Phenolic compounds are one of the secondary metabolites that are produced in plants via shikimate pathway (Heldt 2005). They are presence in plant organs play several structural and physiological roles, including but not limited to the defense, pigmentation, growth and reproduction (Lattanzio et al. 2006). Analysis of phenolic contents has been widely studied for stress detection in terrestrial plants and marine macroalgae. In marine angiosperms, phenolic compounds have been identified in response to different stressors both biotic and abiotic (Agostini et al. 1998, Dumay et al. 2004, Steele et al. 2005). However, such studies are largely limited to temperate species and little has been done on tropical seagrasses in particular those found along the coast of Indian Ocean in Dar es Salaam. Being a big city, Dar es Salaam is facing a problem of population increase leading to an exponential increase in pollutants rich in nitrogen and phosphorus into the coastal zone. Consequently deterioration of seagrass meadows is increasing uncontrolled. Therefore, this study was set out to investigate the influence of seasonal variation on phenolic contents of seagrass *Thalassia hemprichii* from four different sites having different magnitude of anthropogenic nutrient loading along the coast of Dar es Salaam.

MATERIAL AND METHODS

Study site

This study was conducted in four sites along the Indian Ocean coast in Dar es Salaam namely; Ocean road, Mjimwema, Kunduchi, and Oysterbay. Ocean Road is located between 6°48' 1.44" S - 39° 17' 12.48" E and Mjimwema is located between 6° 52' 30" S - 39°20' 6" E. Kunduchi is located between 6° 37' 48" S - 39° 13' 48" E and Oysterbay is located at 6° 46' 30" - 39° 16' 30" E (Fig. 1). The four sites were chosen based on the fact that they are all dominated with the seagrass *Thalassia hemprichii* and are also subjected to different anthropogenic disturbances and receive varied levels of nutrients loading; hence expected to portray different patterns of phenolic contents in the selected seagrass.

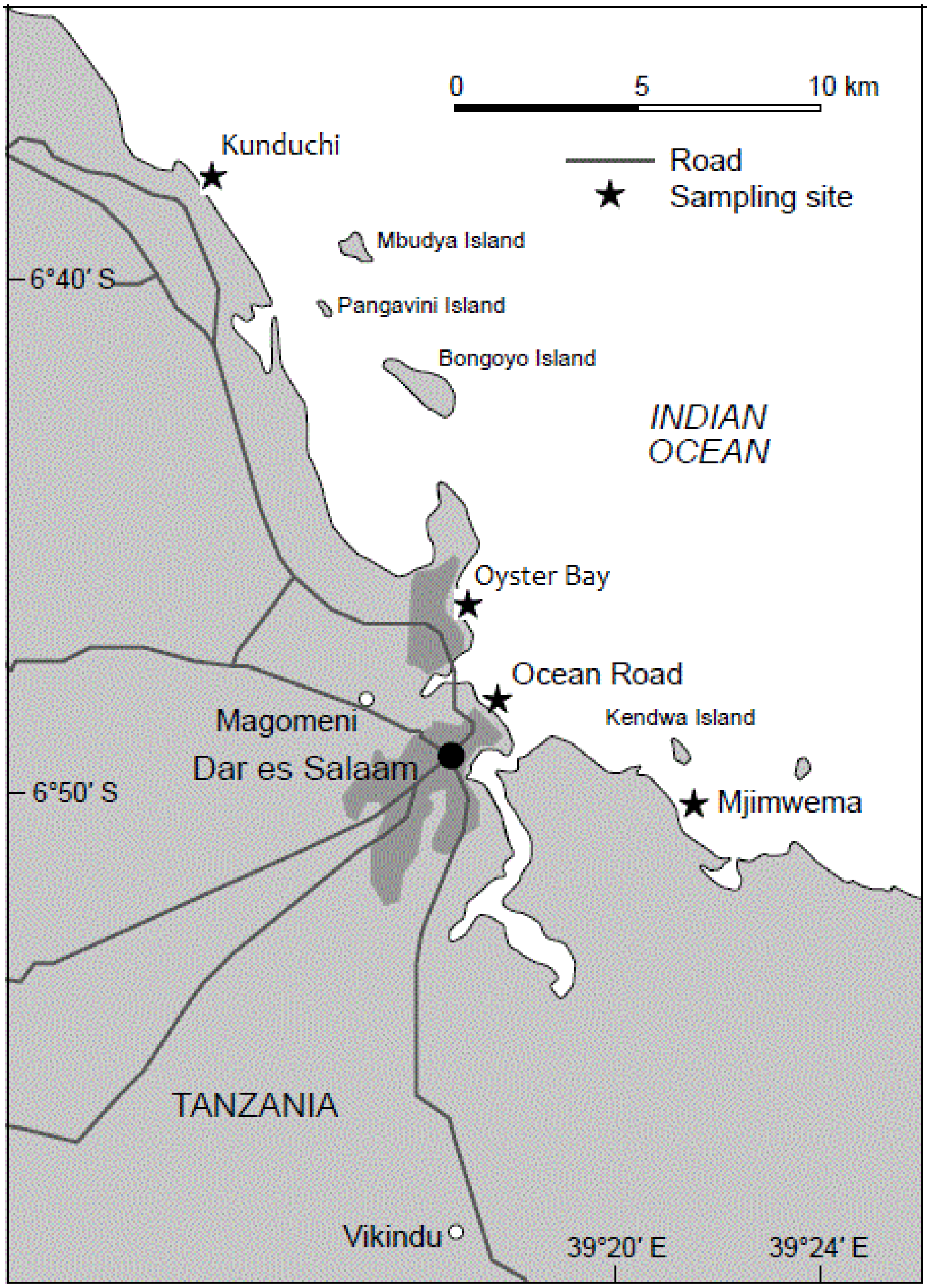

Figure 1. Map of the Dar es Salaam coast showing study sites (solid star). Modified from Mvungi and Mamboya (2012)

Sampling procedure
Sampling was conducted in October 2013 (dry season) and in April 2014 (rainy season). At each sites, ten representative intact *T. hemprichii* individual were sampled by uprooting them using shovel. Because the seagrasses were not evenly distributed and occurred in patches of different sizes, sampling were done opportunistically. The samples were stored in the cool box and then brought to the laboratory at Botany Department of the University of Dar es Salaam for further processing. In the laboratory, seagrass were cleaned with tap water to remove sediments and epiphytes, separated into above and belowground parts then stored in the freezer at -20°C until extraction. The extraction was done within 48 hours after sampling.

In addition to plant materials, during each sampling visit at each site, five water samples were collected from the tidal pools using clean plastic bottles (250 ml) and brought to the laboratory for analysis of nutrient concentration. Upon arrival, samples were filtered through Whatman GF/F filters. The samples were analysed immediately or frozen at -20°C. Determination of nutrient concentration in water was essential in order to know the status of each site and assess its influence on phenolic concentration.

Determination of total phenol contents
The determination of total phenolic content was performed by adapting the Folin-Ciocalteu method (Ainsworth and Gillespie 2007). Briefly, below ground and above ground parts separately were homogenized by using mortar and pestle and were mixed with ice cooled methanol in a ratio of 0.1 g of seagrass and 10 ml of methanol. The sample was incubated at room temperature for 48 hours in the dark. Thereafter, samples were centrifuged at 13,000 g rotation for 5 min at room temperature and supernatants were collected into new clean tubes. Then 200 µl of 10% Folin–Ciocalteu reagent was added, thereafter a total of 800 µl of 700 mM sodium carbonate (Na_2CO_3) was added into each tube and incubated at room temperature for 2 hours. After incubation period, a total of 200 µl sample aliquot, standard or blank were prepared in new tubes and their absorbance were quantified using spectrophotometer (UV-visible Jen way 6305 S UK) at 765 nm. Total phenolics were calculated as gallic acid equivalents (GAE).

Determination of nutrient concentration in the water
The concentrations of nitrogen and phosphorus were determined in triplicate samples following procedures described by Parsons et al. (1989).

Data analysis
The differences in phenolic contents of seagrass *T. hemprichii* from four sites were compared by using one way analysis of variance (ANOVA), followed by Tukey-Kramer multiple comparison tests. To test for the relationship between total phenol contents in the seagrass and nutrients concentration in the water column, Pearson correlation coefficient was used. All the analyses were done using Graph Pad Instant software. *P*. values less than 0.05 were considered significant.

RESULTS
Total phenolic contents
The mean concentration of total phenol in the above ground and below ground parts of *T. hemprichii* in dry season varied from 8.9 ± 5.96 µg GAE / mg to 21.7 ± 9.93 µg GAE / mg, for above ground parts and from 10.5 ±1.78 µg GAE / mg to 84.8 ± 11.38 µg GAE / mg, for below ground parts. The lowest concentration in both above and below ground parts was recorded at Oysterbay and the highest being at Kunduchi. When

comparing all sites sampled during dry season, total phenol concentration in the seagrass tissues varied significantly among sites (ANOVA, P < 0.0001, Table 1, Fig 2). In a general trend, the sites of sample collected during dry season arranged on basis of total phenolic content from lowest are as follows: Oysterbay < Ocean Road < Mjimwema < Kunduchi. Furthermore, in all sites the concentration of total phenol was significantly higher in below ground tissues than in above ground tissues (P < 0.0001), except for Oysterbay where no significant difference in concentration of phenol between the two parts were observed.

Table 1: **Concentration range of total phenol (µg GAE / mg) in *Thalassia hemprichii* recorded in different sites along Dar es Salaam coast in dry and rainy seasons.**

Sites	Dry season		Rainy season	
	Above ground	Below ground	Above ground	Below ground
Ocean Road	13.3 – 20.9	10.7 – 92.9	4.6 – 24.1	15.6 – 49.0
Mjimwema	8.0 – 19.3	30.9 – 97.9	6.2 – 26.4	12.7 – 77.2
Oysterbay	2.7 – 20.9	1.9 – 20.5	6.7 – 17.7	26.0 – 73.3
Kunduchi	6.0 – 35.7	27.9 – 142.9	2.3 – 8.3	24.8 – 70.2

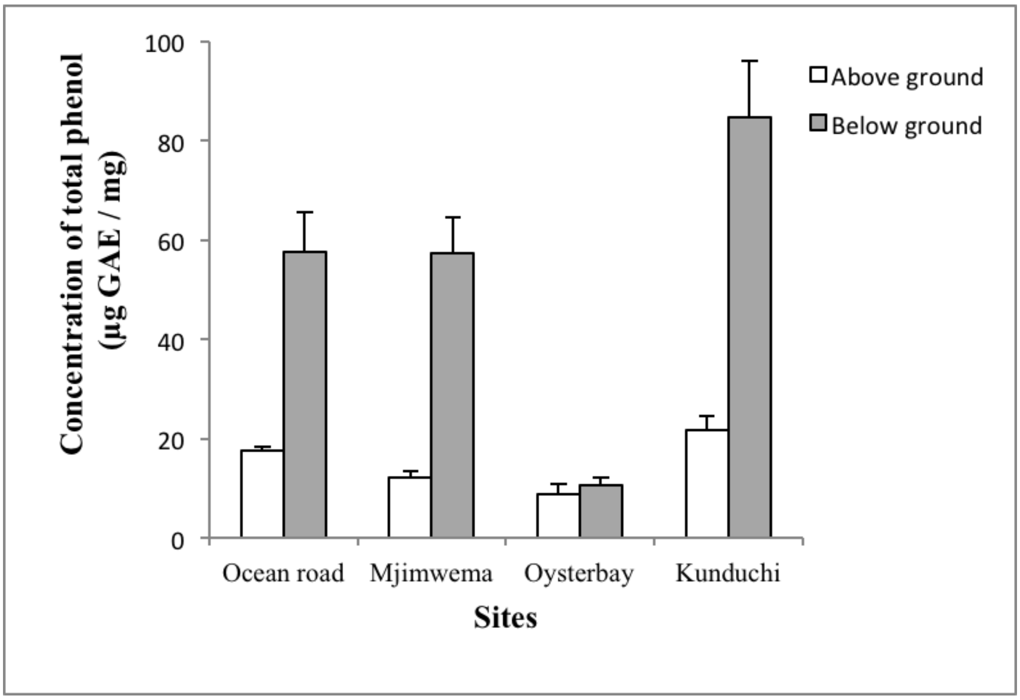

Figure 2: Mean concentration of total phenol in above and below ground parts of *T. hemprichii* collected from four different sites along Dar es Salaam coasts during dry season. Values are mean ± SE, n = 10.

To evaluate seasonal influence on the concentration of total phenol in the seagrass *T. hemprichii*, the assessment was also done during rainy season. The results obtained indicated a significant difference in concentration of total phenol in both above and below ground parts among the sites as well as within site ($P < 0.0001$, $F = 10.0$, Table 1, Fig. 3). Multiple comparison tests on concentration of phenol among sites indicated pairs of means that attributed to the observed differences to be only between the following: Ocean Road and Kunduchi; Mjimwema and Oysterbay; Mjimwema and Kunduchi ($P < 0.05$). Whereas the mean concentration of total phenol in the above ground parts of *T. hemprichii* varied from 5.4 ± 0.58 µg GAE / mg to 17.7 ± 1.94 µg GAE / mg, the minimum being at Kunduchi and the highest being at Mjimwema (Fig. 3), that of below ground parts ranged from 27.5 ± 3.69 µg GAE/mg to 48.5 ± 4.22 µg GAE/mg at Ocean road and Oysterbay, respectively (Fig 3). Similar to the dry season, the phenol content of below ground tissues were significantly higher than above ground tissues ($P < 0.0001$, $F = 18.8$, Fig 3).

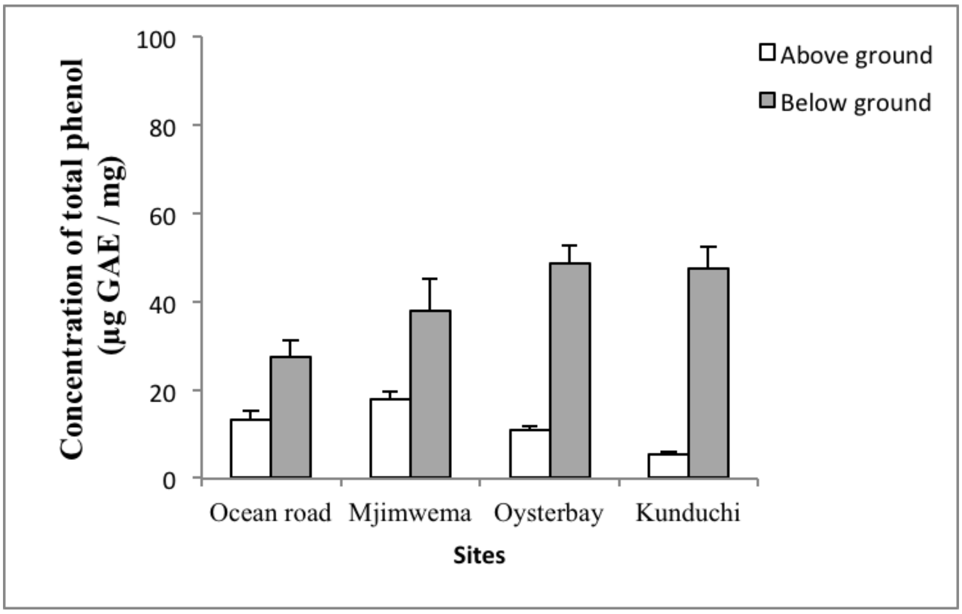

Figure 3: The concentrations of total phenol in above and below ground parts of *T. hemprichii* collected during rainy season in four different sites. Data are means ± SE, n = 10.

The comparison of total phenol concentration in *T. hemprichii* between the two seasons done using unpaired t-test in each individual site showed that the significant variation in total phenol concentration between the two seasons were found in all sites ($P < 0.05$) except in Mjimwema. The general trend shows that the higher concentration of total phenol was found during dry season with exception to Oysterbay where the pattern was opposite (Fig 2 and 3).

Relationship between nutrients in water and phenolic concentrations

The concentrations of nitrates and phosphorus in water were analyzed by one way analysis of variance during the dry and rainy seasons. There was significant seasonal variation in nitrate concentration within and among the sites. The highest nitrate concentration was recorded during rainy season in all sites as compared to dry season (F = 12.7, P < 0.0002, Table 2). Similarly, the concentration of phosphorus varied significantly among sites and the higher concentration was recorded in rainy season at three sites except Ocean Road which showed opposite trend (F = 6.012, P < 0.0061, Table 2).

Table 2: **Concentration of nitrate and phosphorus in the water column collected from four different sites along the Dar es Salaam coast during rainy and dry seasons**. (OR = Ocean Road; MJ = Mjimwema; OY = Oysterbay; KN = Kunduchi).

	Dry season				**Wet season**			
	OR	MJ	OY	KN	OR	MJ	OY	KN
Phosphorus (µM)	0.074	0.096	0.15	0.058	0.13	0.17	1.06	0.28
Nitrate (µM)	0.70	0.34	0.18	0.13	0.47	0.57	0.34	3.34

The relationship between levels of nutrients in water column and the total phenolics in plants was done using correlation analysis. There was negative correlation between level of nutrients in water and the concentration of total phenol in the seagrass organs (r = - 0.95).

DISCUSSION

Plants produce secondary metabolites in the course of their normal growth and development, and for defensive purpose to deter animals feeding on them as well as against environmental disturbances. Phenolic compounds like any other secondary metabolites are usually present in plant tissues at different concentration and play important role in plant defense, antioxidants and free radical scavengers (Soobrattee et al. 2005) just to mention but a few. In this study total phenol in tissues of seagrass *T. hemprichii* from four different sites along Dar es Salaam coast were determined during dry and rainy seasons, in order to assess whether there is seasonal variation as well as spatial distribution of the same. The highest concentration of total phenol was recorded during dry season in all sites except Oysterbay which showed opposite pattern.

Secondary metabolites represent a chemical interface between plants and their environment, therefore their synthesis is often affected by prevailing environmental conditions (Kutchan 2001). For example, high environmental nutrient concentration have been shown to affect levels of phenolics in marine flora (Buchsbaum et al. 1990, Yates and Peckol 1993, Cronin and Lodge 2003, Dethier et al. 2005) and also may affect other aspects of resource allocation as growth, reproduction, and chemical defense (Pfister and Van Alstyne 2003).

When comparing between above and below ground tissues of *T. hemprichi*, below ground tissue had significant higher total phenol than that of the above ground tissues in all seasons except at Oysterbay in dry season where the pattern was opposite (Fig. 2). This higher total phenol in the below ground tissue than the above ground tissue could be due to the fact that rhizomes has long life span in such that, they live for a long time than the leaves and probably they could have experienced environmental perturbations for a long time compared to the leaves which are ephemeral in most cases surviving for several months before they fall off. This observation is further supported by other researchers who did related works. For example Rotini et al. (2011), Dumay et al. (2004), Migliore et al. (2007) explained how total phenol concentration varies with season and also indicated that levels of synthesis and accumulation of phenolic compounds are more stable in rhizomes due to their long life span which allows the maintenance of memory from previous environmental pressures.

The observed high total phenol at Kunduchi in both leaves and rhizomes in dry season is connected to the effects of nutrients and partly might be caused by other anthropogenic activities evidenced during the sampling occasions for example the oil spill from the old ship which was stationed near the seagrass meadow releasing oil pollutants to the extent that high percentages of seagrasses around that area were bleached as a sign of oil spill stresses, also reported in Juday and Foster (1990).

Furthermore, the observed variation in phenol contents in this study could be partly contributed by the variation in nutrient concentrations (Table 2). Availability of nutrients such as nitrogen and phosphorus in the marine environments has been a major attribute that could cause variation on the accumulation of the phenolics and other secondary metabolites in the seagrass (Buchsbaum et al. 1990). In this study significant inverse relationship between concentration of nutrients and phenol contents in seagrass was observed. It is known that the allocation of resources in plants depends on the nutrients availability in the environment where they grow. Usually under adequate supply of nutrient plants allocate more carbohydrate to growth rather than production of defensive compounds, while under nutrients limitation plants allocate more resources for the synthesis of defensive chemicals or secondary metabolites such as phenolics. This scenario is congruent to the observed patterns in this study as well as other researchers (e.g. Cronin and Lodge (2003), Jormalainen et al. (2003), Dethier et al. (2005), Cannac et al. (2007).

In addition, significant seasonal variation in accumulated total phenols in *T. hemprichii* from different sites was observed. Total phenols were found to be high in the dry season for all sites than in the rain season, the pattern which could be attributed by the influence of variable multiple environmental pressures (although were not quantified in the course of this study), including but not limited to change in temperature, rainfall and nutrients acting in concert or at different time. This is in agreement with the study by Connan et al. (2004) who highlighted that high phenolic was produced in the site with high temperature as a result of solar radiation.

Rainfall on the other hand, usually carry different land based materials to the ocean ultimately lead to changing water body conditions and resulting to the changes in nutrients status and probably on the pattern of production of phenolic compound in the marine vegetation (Cordiova et al. 2013). Many researchers have explained the relationship between phenol content and environmental pressures that have been previously observed in several seagrass meadows exposed to different environmental stresses such as pollutions, ocean acidification, nutrient enrichment, metal contamination and epiphytes (see Dumay et al. (2004), Migliore et al. (2007), Rotini et al. (2011), Arnold et al. (2012), Ferrat et al. (2012). In their study on phenolic compounds in aquatic plants and filamentous algae, Joaquim et al. (2008) verified that the amount and types of secondary metabolites varied among the species studied depending on their habitats, plant organs, and seasons of the year. This is in line with the observed patterns in the present study, as the variations were observed among sites as well as within sites, indicating heterogeneity in the area where seagrass *T. hemprichii* grow.

CONCLUSION

In this study we observed that analysis of biomarkers such as phenolic contents could be used as an indicative measure of stressors of seagrasses under different environmental perturbations. The findings of the present study indicates significant variation in phenolic content in *T. hemprichii* among studied sites which portray clearly the heterogeneity behavior of these sites and suggest that they face different pressures especially nutrient levels and have different properties which provide variable microhabitat to the studied seagrass and hence observed variation in the accumulation of phenolics depending on the site. Furthermore, it was observed that the seasonal and nutrients variation have much influence on the concentration of total phenol in the studied seagrass species. Thus calling for other study that could focus on other species of seagrass found in the same area in order to have a holistic understanding of phenolic contents pattern in seagrass species found along the whole of Dar es Salaam coast and thus qualify it as one of the potential early warning biological markers of environmental perturbation.

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