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Effect of Seawater and Surface-Sediment Variables on Epipelic Diatom Diversity and Abundance in the Coastal Area of Negeri Sembilan, Malaysia

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Abstract: Benthic diatoms are important components of marine shallow-water habitats that may affect primary production, stabilize sediment, and produce extracellular polymeric substances. Benthic diatoms are useful for estimating the trophic status of marine ecosystems. In this study, we investigated the diversity and abundance of benthic diatoms to integrate these data with the physicochemical characteristics of shallow coastal areas in Negeri Sembilan. A total of 39 species of epipelic diatoms were extracted by removing organic matter from sediments that were dominated by pennate diatoms. Results showed that *Diploneis crabro*, *Eunotogramma laevis*, *Actinoptychus* sp., and *Cocconeis placentula* were the important species in the area. The abundance varied between 1.85×10^3 and 3.43×10^3 cells/g, and the diversity index fluctuated between 2.13 and 2.58. The abundance had significant positive correlations with seawater surface temperature (SST) but had negative correlations with pH and NH_3. The diversity on the other end was positively correlated with SST but negatively correlated with total suspended solids and SiO_2. Principal component analysis (PCA) demonstrated that the abundance of *D. crabro*, *E. laevis*, and *Actinoptychus* sp. can be attributed to high levels of NO_2^-, NH_3, and total dissolved solids. PCA also showed positive correlations of *C. placentula* with NO_3^- and SiO_2 but negative ones with PO_4^{3-} and pH. The epipelic diatom community showed high diversity with high variations throughout the study area.

Keywords: benthic diatom; species; pennate; variations; Negeri Sembilan

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1. Introduction

Coastal areas are highly productive regions of the ocean, with high contributions from planktonic and benthic primary production. Although coastal areas and estuaries constitute less than 10% of the ocean, they contribute up to 30% of the ocean's primary production [1,2]. They serve as important nursery grounds for fish larvae, habitats for benthic organisms, and feeding grounds for many marine animals [3]. Coastal areas are also the epicenter of human settlement and activities, where almost three-quarters of the world's human population resides. Consequently, an unprecedented increase in nutrients and other environmental issues associated with coastal development has occurred [4]. Problems such as nutrient over-enrichment and eutrophication of estuarine and coastal ecosystems are common and accelerating [4].

Diatoms (Class Bacillariophyceae) constitute the main mass of marine phytoplankton and have a worldwide distribution, with recent estimates ranging from 12,000 to 30,000 species, contributing around 20% of the total phytoplankton primary production [5,6]. Diatoms in estuaries or shallow marine systems can be classified into two groups, namely the benthic and pelagic groups. The former can be resuspended into the water column by turbulence [7]. Epipelic diatoms often dominate the microphytobenthos, which is important for the primary productivity of the benthic zone [8]. Their diversity and composition can be influenced by wide-ranging environmental variables [9,10], and their growth forms show a distinct distribution among intertidal habitats characterized by different types of sediment [11,12].

Physicochemical variables such as water temperature, salinity, nutrients, pH, and DO are among the most important factors controlling phytoplankton growth, diversity, and production in marine environments [13,14]. Epipelic diatoms provide many benefits in the coastal area, such as being a source of primary production and the main food source for microherbivores. They can also be used for biological monitoring because they lie at the base of aquatic food webs and are among the first rapid response to the environmental stress of organisms [10,15]. Despite their ubiquity and functional importance, the spatial and temporal patterns of the abundance and diversity of epipelic diatom groups are poorly understood. In Malaysia, the taxonomic composition of intertidal epipelic diatom communities remains relatively unknown. Conversely, the phytoplankton taxonomy of Malaysia has been studied in detail over the years [16]. Previous studies on phytoplankton in the Malacca Straits have been conducted by several authors [16–25]. They have shown that diatoms are highly dominant in plankton and contribute as main actors in the pelagic realms. Diatoms may also be the dominant group in the benthic area, but this aspect has rarely been reported within the Malacca Strait areas. Benthic diatoms are indeed a very important component of coastal and estuarine systems and represent a key component in the primary production of these coastal habitats. They are responsible for up to 30% of carbon fixation of those ecosystems, so they are suppliers of organic compounds to grazers to deposit feeder's aquatic organisms, including macro- and meiofauna. Accordingly, the current research aims to fill the gaps and contributes to the knowledge of epipelic diatom diversity and abundance in coastal habitats, as well as to evaluate the role of physicochemical variables in affecting the epipelic community composition in the intertidal zone of the Port Dickson Coast of Negeri Sembilan, Malacca Straits area, Malaysia.

2. Materials and Methods

2.1. Study Area

The study area was located within the coastal area of Port Dickson, in Negeri Sembilan, Malaysia. The coast is about 54 km long and faces the Straits of Malacca. Field samplings were conducted in December 2019, during low-tide periods. Surface sediment samples were collected from six sampling stations (denoted as St.1 to St.6) located within the intertidal zones, with five replicate samples at each station. These five samples were collected from each site and composited into one homogeneous sample representing the station (Figure 1). The epipelic diatoms were sampled using a PVC core of 8.4 cm diameter, and sampling was performed on the same day, with a time difference of less than half an hour between stations. The top 1 cm layer of wet and exposed surface sediments at the edge of the seawater was collected. The sediments containing epipelic diatoms were placed in a black polythene bag and maintained in darkness in a refrigerator until processing in the laboratory [26].

2.2. Epipelic Diatom Extraction and Counting

Epipelic diatoms were collected and extracted according to the method described by [27,28]. Around 1 g of wet weight of surface sediment was heated at 70 °C with 30% hydrogen peroxide (H_2O_2) and 10% HCl in a water bath until all organic matter and carbonates were digested. The sediment was subsequently washed with deionized water and left to settle to remove the acids. Around 0.5 mL of cleaned sample was transferred to a cover slip and air dried on a warm hotplate. Three prepared slides from each sample were counted for the epipelic species, resulting in three replicate abundance estimates.

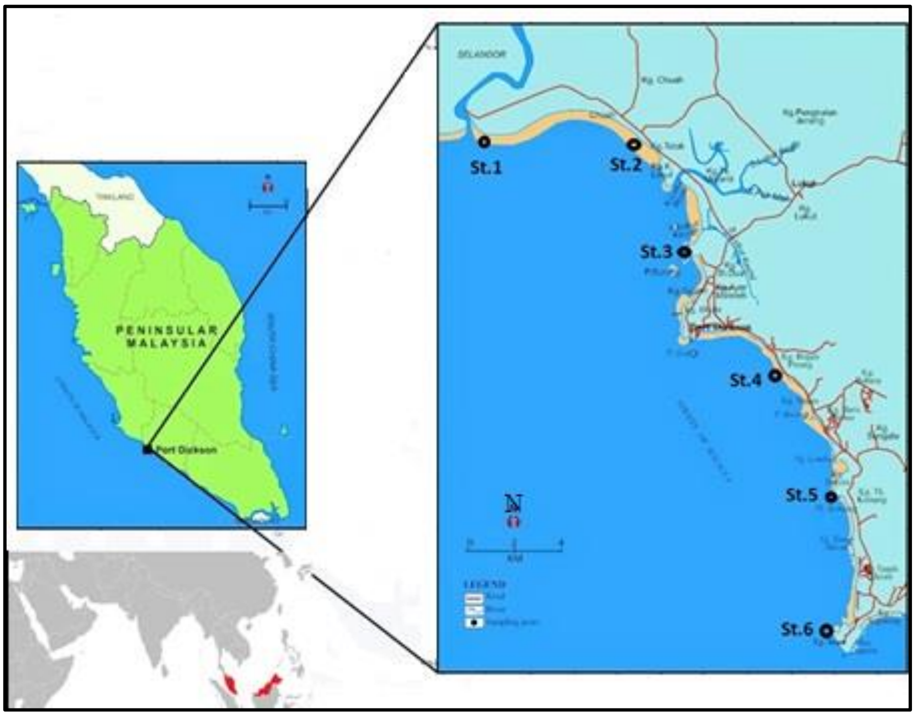

Figure 1. Study area located along the Port Dickson coast, Malaysia, stretching from Sungai Sepang in the north to Tanjung Tuan in the south. Circles indicate the sampling stations.

Counting and identification were conducted under a compound light microscope (Leica DM1000 LED, Wetzlar, Germany) with a counter chamber (Sedgwick-Rafter, Graticules Optics Limited, Cambridge, UK). Identification was based on previous descriptions [26,29–31]. Epipelic diatom diversity and richness were calculated using the Shannon–Wiener index [32] and Margalef's index [33].

2.3. Environmental Parameters

Surface seawater temperature (SST), surface seawater salinity (SSS), dissolved oxygen (DO), electrical conductivity (EC), total dissolved solids (TDS), and pH were measured in situ with a handheld GPS Aquameter (AP 700, Bath, UK). Around 3 L of seawater samples were collected in a plastic container from the intertidal zone and immediately kept in a cool condition before transporting back to the laboratory for nutrient analysis. Nitrate (NO^{3-}), nitrite (NO^{2-}), ammonia (NH_3), silica (SiO_2), and phosphate ($PO_4{}^{3-}$) were analyzed using a HACH DR2010 spectrophotometer (HACH Company, Loveland, CO, USA). Total suspended solid (TSS) concentrations were determined by a previously described method [34]. For chlorophyll-a analysis, the seawater samples were passed through a GF/F filter paper (Whatman GF/F-F4-4700, Maidstone, UK), which was then covered with aluminum foil and placed in a deep freezer (Haier DW-40L262, Qingdao, Shandong, China) in darkness at −20 °C until extraction with 10 mL of 90% acetone [35]. Chlorophyll-a was determined with a spectrophotometer (Shimadzu UV/VIS mini-1240, Kyoto, Japan). Results were compared with the Malaysian Marine Water Quality Standards (MMWQS) published by the Department of Environment, Malaysia [36]. Sediment organic matter (OM) was estimated by the percentage loss on ignition technique as described by [37]. A half-gram of wet sediment was oven dried for ~24 h at 90 °C (Memmert universal oven UN30, Büchenbach, Baden-Württemberg, Germany) to a constant weight. The remaining dry sediment was then combusted in a muffle furnace (Daihan scientific co. ltd., Gangwon, South Korea)) at 550 °C for 4 h for complete ignition of the OM. After ignition, the sediment samples were cooled in a desiccator, and the weight loss (% dry weight) was determined.

2.4. Data Analysis

Statistical data analysis (Pearson correlation coefficient) was performed using SPSS 20.0 (IBM, Armonk, NY, USA). To characterize physicochemical variables and their influence on epipelic diatoms in the study stations, principal component analysis (PCA) was performed using a dataset of 14 seawater parameters and epipelic diatom abundance data in the study area.

3. Results

3.1. Environmental Conditions

The spatial variations in physicochemical variables along the coastal area are summarized in Figures 2 and 3. In general, temperature variations in the coastal waters of the Port Dickson coast are small, ranging from (28.73 ± 0.26) °C in St.3 to (31.19 ± 0.28) °C in St.5. However, the variations in SSS levels are high, ranging from (20.20 ± 0.26) ppt in St.3 to (27.33 ± 0.35) ppt in St.5. The pH ranged from 7.72 ± 0.30 in St.1 to 8.34 ± 0.38 in St.2, and the DO ranged from (6.72 ± 0.33) mg/L in St.5 to (7.74 ± 0.30) mg/L in St.1. The EC was relatively consistent, ranging from (26,220.67 ± 27.08) µS/cm in St.1 to (30,853.75 ± 18.6) µS/cm in St.6. The TDS also showed high variations, ranging from (18,532.67 ± 13.7) mg/L in St.6 to (19,420.33 ± 15.31) mg/L in St.4, whereas the TSS fluctuated between (45.4 ± 0.83) mg/L in St.4 and (77.91 ± 0.95) mg/L in St.1. The sediment OM varied between 21.00 ± 0.8% and 25.85 ± 0.49%, with maximum values in St.6 and minimum in St.2 (Figure 2).

Among the nutrients, NO_3^- ranged from (0.018 ± 0.0014) mg/L in St.5 to (0.033 ± 0.0007) mg/L in St.3. The NO_2^- concentrations were relatively lower, ranging from (0.002 ± 0.0006 and ± 0.0012) mg/L in St.5 and St.6, to (0.01 ± 0.007) mg/L in St.2. NH_3 ranged from (0.38 ± 0.021) mg/L in St.6 to (0.53 ± 0.014) mg/L in St.3, which was much higher than the nitrate + nitrite levels. PO_4^{-3} concentrations were not as pronounced as the ammonia, with the highest concentration recorded in St.5 (0.21 ± 0.011) mg/L and the lowest in St.2 (0.04 ± 0.002) mg/L. SiO_2 ranged from (0.08 ± 0.011) mg/L to (0.11 ± 0.014 and ± 0.012) mg/L, with the lowest concentrations in St.1 and highest in St.2 and St.5. The range of concentration for Chl-a in the six stations was from 0.10 ± 0.028 mg/L to 0.13 ± 0.021 mg/L, with the highest concentration recorded at St.6 and the lowest at St.5 (Figure 3).

3.2. Dynamics of Epipelic Diatoms

A total of 39 epipelic diatom species were collected and identified, and the pennate diatoms (78% at St.5) were more dominant than the centric ones (47% at St.1) (Figure 4a). The overall diatom abundance ranged from (1.85 × 10^3 ± 0.09 cells/g) in St.3 to (3.43 × 10^3 ± 0.18 cells/g) in St.6 (Figure 4b). The Shannon–Wiener diversity index (H') was relatively high, ranging from 2.13 in St.1 to 2.58 in St.4, whereas the Margalef's richness index ranged from 1.32 in St.2 to 2.08 in St.5.

The percentage composition of diatom species recorded at each station is summarized in Table 1. *Cocconeis placentula* and *Eolimna minima* were the most common species, with 67% occurrence. Notably, each station was dominated by different species, where *C. placentula* was dominant in St.1 (41%), *Diploneis crabro* in St.2 (24%), *Eunotogramma laevis* in St.3 (34%), *Actinoptychus* sp. in St.4 (15%), *Amphora* sp. in St.5 (28%), and *Coscinodiscus* sp. in St.6 (15%) (Table 1 and Figure 5). These results indicated high spatial variations in epipelic distribution along the stations.

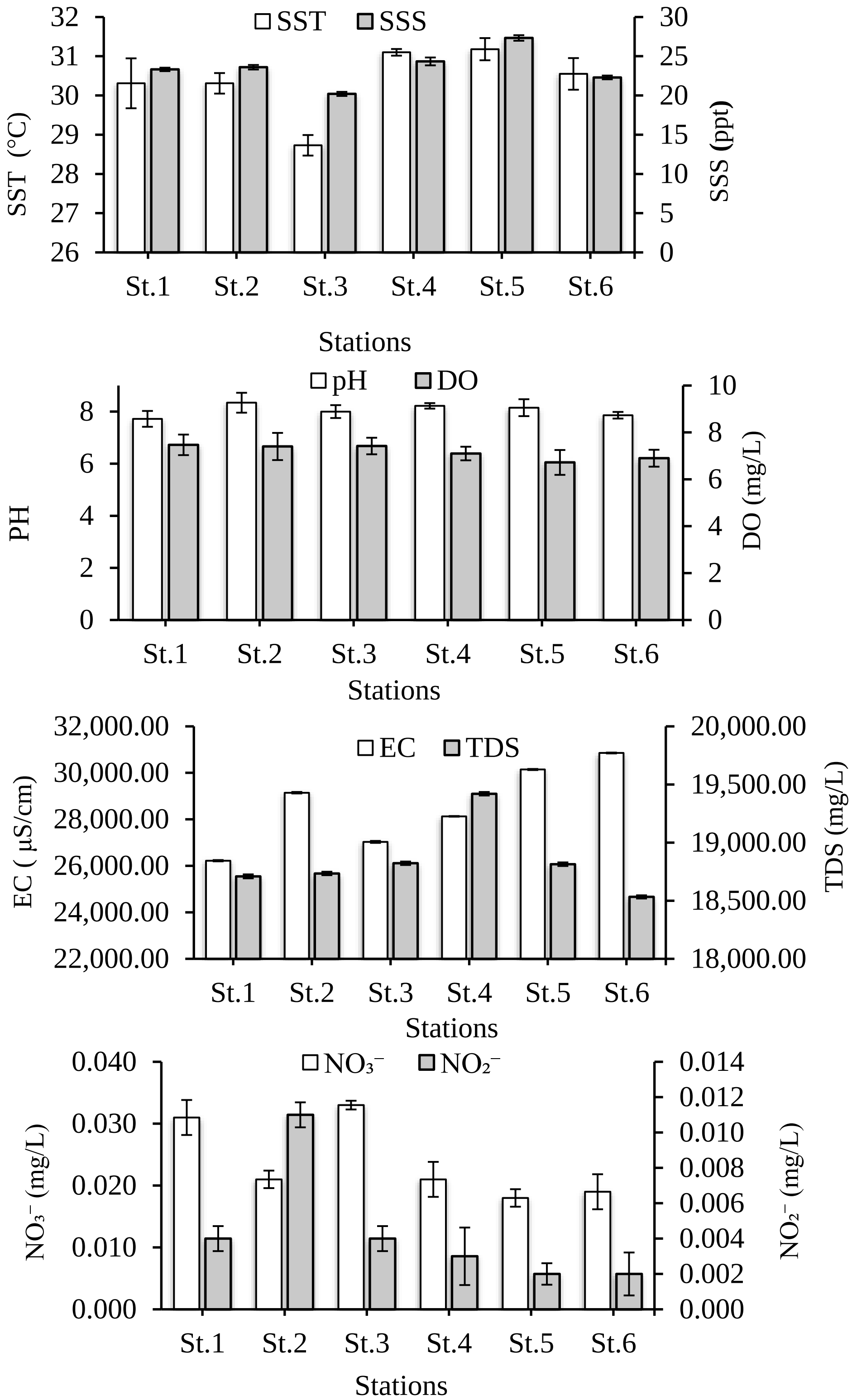

Figure 2. Variations in seawater parameters (mean ± SD) along the study area: seawater surface temperature (SST) and salinity (SSS); pH and dissolved oxygen (DO); electrical conductivity (EC) and total dissolve solid (TDS); and total suspended sediment (TSS) and organic matter (OM).

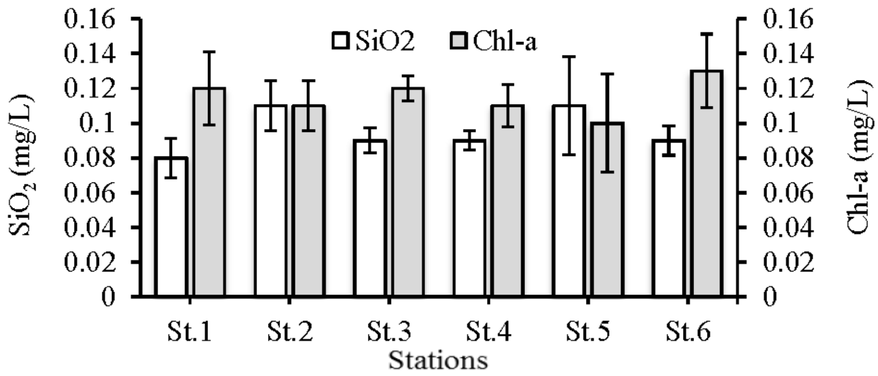

Figure 3. Variations in seawater nutrients (mean ± SD) along the Port Dickson coasts, Malaysia: nitrate (NO_3^-) and nitrite (NO_2^-); ammonia (NH_3) and phosphate (PO_4^{-3}); and chlorophyll-a (Chl-a) and silica (SiO_2).

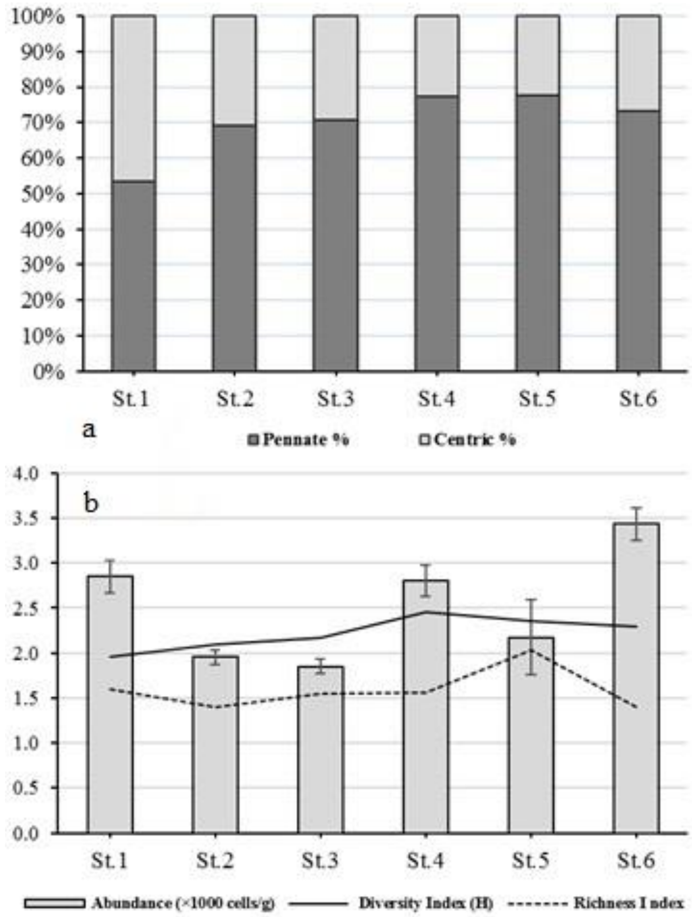

Figure 4. Epipelic diatom population parameters along the study area. (**a**): Percentage contribution of pennate diatom and centric diatom; (**b**): Abundance, diversity (Shannon–Wiener index) and richness (Margalef's index).

Table 1. List of epipelic diatoms species predominance (%) at the sampling stations along Port Dickson coast, Malaysia. Occurrence (%Pr): 0–20 (sporadically, S); 21–40 (rarely, R); 41–60 (commonly, C); 61–80 (frequently, F); and 81–100 (highly frequently, H).

	St.1	St.2	St.3	St.4	St.5	St.6	%Pr	Class
Actinoptychus sp.	9		3	15			50	C
A. undulates (J.W.Bailey) Ralfs, 1861.			3		3	5	50	C
Amphora arenaria Donkin, 1858.		13	8		4		50	C
Amphora sp.	2			5	28		50	C
Auliscus elegans Auliscus elegans var. californica (Grunow in Schmidt et al.) Rattray, 1888.	4	5		8			50	C
Caloneis sp.					8	3	33	R
Campylodiscus sp.	7	3				6	50	C
Cocconeis placentula Ehrenberg, 1838.	41	14		5	1		67	F
C. radiatus Ehrenberg, 1840.						12	17	X
C. gigas var. praetexta (Janisch) Hustedt, 1930.	7	9	11				50	C
Coscinodiscus sp.	6					15	33	R
Cyclotella striata Grunow in Van Heurck, 1882.			6	8	13		50	C
Diploneis crabro Ehrenberg, 1854.		24	5		2		50	C
Diploneis obliqua (Brun) Hustedt, 1937.		11					17	X
Eunotogramma laevis Grunow, 1883.			34		5	6	50	C
Eolimna minima (Grunow) Lange-Bertalot & W.Schiller, 1997.	5		6	8		7	67	F
Gyrosigma eximium (Thwaites) Boyer, 1927.	3				6		33	R
Lyrella clavata (Gregory) D.G.Mann, 1990.				10			17	X
Lyrella sp.		5	2				33	R
Mastogloia angulata Lewis, 1861.	5		6		4		50	C
Melosira sp.			3		3	11	50	C
Navicula sp.				4			17	X
N. longa (Gregory) Ralfs ex Pritchard, 1861.			9				17	X
N. peregrine	4				4		33	R
Nitzschia sigma (Hantzsch) Grunow, 1878.				7			17	X
Odontella sp.				5		10	33	R
O. mobiliensis (J.W.Bailey) Grunow, 1884.					3		17	X
Paralia sulcate (Ehrenberg) Cleve, 1873.			5		1		33	R
Petroneis granulate (Bailey) D.G.Mann, 1990.	4			8	1		50	C
Pinnularia sp.						7	17	X
P. aestuarii Cleve, 1895.		3					17	X
Pleurosigma sp.		10				4	33	R
P. naviculaceum Brébisson, 1854				4		4	17	X
pseudo-nitzschia sp.	2						17	X
Surirella sp.				7			17	X
S. fastuosa (Ehrenberg) Ehrenberg, 1843.					6		17	X
S. spiralis Kützing, 1844	2						17	X
Thalassiosira sp.		3		8	8		50	C
Triceratium sp.						13	17	X

The correlations between the epipelic diatom's abundance and diversity against various environmental parameters are presented in Table 2. Significant positive correlations existed between abundance of epipelic diatom taxa against SST and TSS ($p < 0.05$), and significant negative correlations existed among the abundance of the epipelic diatom community and pH and NH_3 ($p < 0.05$). Meanwhile, the epipelic diversity showed a significant negative correlation with TSS and SiO_2 and a significant positive correlation with SST ($p < 0.05$). Further analysis using PCA showed eigenvalues of 6.20 and 2.80, respectively, which explained 79.83% of the variance (Figure 6). The abundance of *D. crabro*, *Actinoptychus* sp., and *E. laevis* in St.2, St.4, and St.3 were positively correlated with NO_2^-, DO, NH_3, and TDS and negatively correlated with OM and SSS. *Amphora* sp. in St.5 was positively correlated with SST, EC, Chl-a, PO_4^{3-}, and pH but negatively correlated with SiO_2, NO_3^-, and TSS. *Coscinodiscus* sp. in St.6 was positively correlated with SSS and OM.

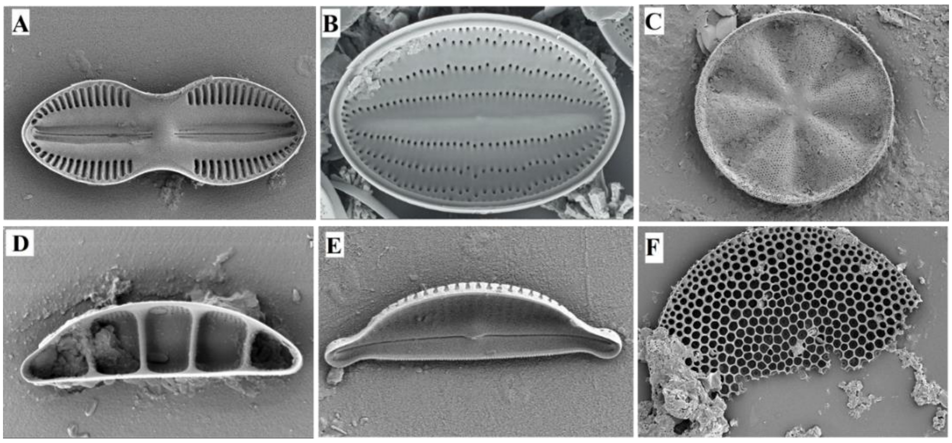

Figure 5. Scanning electron micrographs of the most common species in different stations: (**A**) *Diploneis crabro*, (**B**) *Cocconeis placentula*, (**C**) *Actinoptychus* sp., (**D**) *Eunotogramma laevis*, (**E**) *Amphora* sp., and (**F**) *Coscinodiscus* sp.

Table 2. Pearson's correlation coefficient (r and *p* value) of epipelic diatom abundance (cells/g) and diversity (H′) against significant physicochemical variables at Port Dickson coast. Asterisk (*) indicates significance at 0.05 level (2-tailed). Parameters with no significant correlations were excluded.

Physicochemical Variables	Abundances of Epipelic Diatom Communities (cells/g)		Diversity of Epipelic Diatom Communities (H′)	
	r	*p* Value	r	*p* Value
SST (°C)	0.63	0.03 *	0.58	0.04 *
pH	−0.58	0.04 *	0.43	0.16
TSS (mg/L)	0.53	0.08 *	−0.85	<0.01 *
NH_3 (mg/L)	−0.67	0.02 *	0.07	0.83
SiO_2 (mg/L)	0.36	0.25	−0.63	0.03 *
Chl-a (mg/L)	0.74	<0.01 *	−0.11	0.73

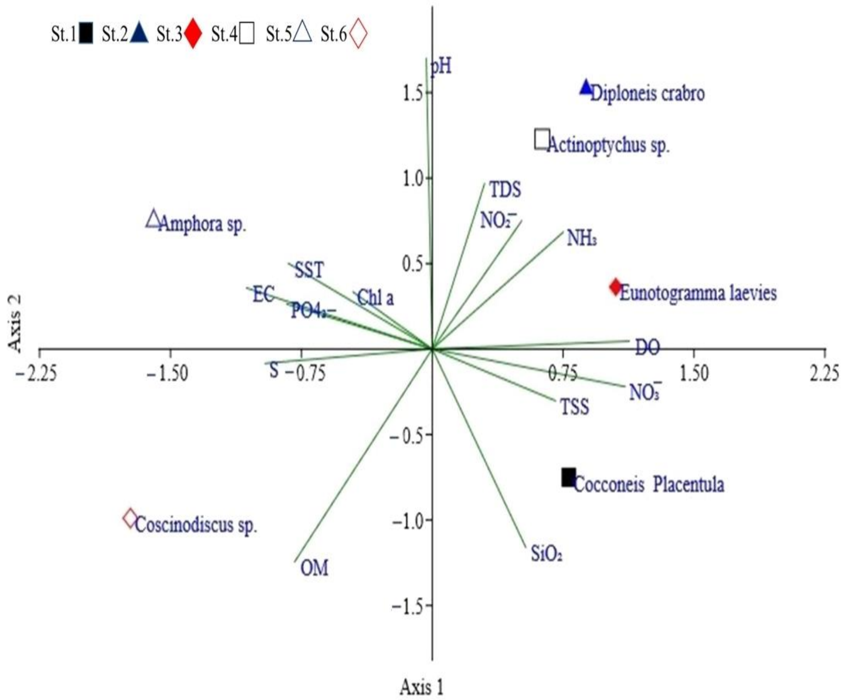

Figure 6. Principal component analysis ordinations of the dominant epipelic diatom species and physiochemical variables measured at six stations along the Port Dickson coasts, Malaysia.

4. Discussion

4.1. Environmental Conditions

Physicochemical variables were measured to determine the coastal-water quality parameters that may affect the epipelic diatom distribution in the different study stations. The SST values were relatively high and stable, with a mean value of 30.36 °C ± 0.89 °C, which is the standard for tropical coastal waters [38]. Increasing temperature can lead to changes in the distribution patterns of benthic diatoms [39]. This phenomenon was found in the current study, where the highest number of epipelic species was recorded at St.5 with the highest temperatures, whereas the opposite was at St.3. Conversely, the SSS levels showed a wide range, which was also normal for nearshore coastal waters. SSS generally did not show any effect on epipelic abundance as it was not among the critical parameters determining the distribution of abundance of epipelic species [40].

pH is an important factor affecting the proliferation of aquatic organisms, and increasing or decreasing pH may affect phytoplankton growth [41]. The pH values recorded in these studies ranged from neutral to alkaline (mean = 8.07 ± 0.21), which were within the MMWQS [36]. The DO values were relatively high, with a mean value of 6.95 ± 0.74 mg/L, similar to a previous study [42]. Variability in the DO levels near the coastline can be attributed to different river outflows along the study area.

The value of TSS in this study can be categorized as under Class III of the MMWQS [36]. St.1 had higher TSS than the other stations, most likely owing to its proximity to the Sepang River. Tidal fluctuations, wind directions, wind speeds, and river outflows were among the major factors regulating the spatial and temporal variations of TSS [43,44]. Other coastal features such as nearby mangroves and coastal vegetations, as well as the amount of OM in coastal waters, may also affect the TSS. The highest OM was recorded at St.6 followed by St.1, where St.6 was located in front of the mangroves, whereas St.1 was located close to the estuary. Studies have shown that mangrove soils may supply significant amounts of OM with high percentages of organic carbon to nearby coastal waters [45,46].

Nutrient concentrations significantly impact phytoplankton occurrence and abundance as they draw in significant amounts of nutrients from the ecosystem [41,47]. However, the present study showed low variabilities in the concentration of nutrients such as NO_3^-, NO_2^-, NH_3^-, PO_4^{3-}, and SiO_2 throughout the stations. Nevertheless, high ammonium concentrations were recorded, as also reported by [48], indicating a high level of pollution in the study area. Furthermore, Ref. [42] reported a high level of pollution in the Port Dickson coasts, and these areas have even been suggested to be unhealthy for human activities.

SiO_2 and Chl-a also significantly affected the abundance of epipelic diatom assemblages and diversity. The SiO_2 concentrations were low at St.1, St.3, St.4, and St.6, but they were relatively higher at St.2 and St.5. Conversely, Chl-a was relatively similar between stations, ranging from 0.10 mg/L to 0.13 mg/L. Chlorophyll is an indicator of biomass variability and phytoplankton growth, and its concentration may be greatly influenced by nutrients [7,49].

4.2. Dynamics of Epipelic Diatoms

The abundance of epipelic diatom recorded throughout the stations was considered as relatively low, which can probably be attributed to the different spatial variables, the high level of pollution, and the poor sediment condition. This finding may have a strong impact on diatoms growing on the sediment surface [50]. Each different group of nutrient concentrations was characterized by a different benthic diatom composition. The relative abundances of benthic diatom forms changed in response to minor inputs of nutrients [51]. The input of OM also caused the addition of suspended solids and the deoxygenation of water. Nevertheless, pennate and centric diatoms were well presented in all stations. The composition of centric diatoms was significantly lower, which was normal because most of them were plankton that adapted to move upwards toward the sediment surface under moderate light intensities and migrated deeper into the sediment in darkness and under very high light intensities [52]. Indeed, the higher ratio of pennate to centric forms is common in the coastal benthic diatoms community and has been previously reported elsewhere [53].

The abundance and diversity of epipelic diatoms showed different correlations with various physicochemical factors. Increase in seawater temperature usually led to higher metabolic activity, thereby increasing the benthic algal biomass [54,55]. Furthermore, OM is important in controlling diatom communities and their nutritive values [26,56]. Previous studies have indicated that variations in OM content play an important role in the diversity of benthic diatom communities, where increasing diatom diversity normally coincides with higher OM content in the sediment [57].

Silica was found to be negatively correlated with epipelic diversity, which may be due to the intensive uptake by some group or species. Previous studies have shown that the silica requirement of epipelic diatom negatively affects the silica balance in the marine ecosystem [58]. Nitrogenous substances are important nutrients for primary productivity. However, Ref. [59] reported that diatoms prefer nitrate but do not respond well to ammonium. Nevertheless, other studies have shown that ammonium is a more readily assimilated source of nitrogen compounds in marine epipelic diatoms, and it is the most important factor determining the sources of the epipelic community structure [40,60]. This phenomenon may result in a shift in their community composition, which explains the negative correlation of ammonia with the abundance of epipelic diatom.

Within the study area, *C. placentula* was one of the most abundant and extensively distributed species. This finding agreed with other studies that also reported *Cocconeis* sp. as the dominant benthic species associated with epiphytic or epipelic habitats [9,61]. *Cocconeis* spp. also contributed as the most abundant benthic microalgae (at 58%) in sediments collected from Muka Head Jetty, Penang, Malaysia [20].

PCA demonstrated that the positive correlation for *D. crabro*, *E. laevis*, and *Actinoptychus* sp. in stations St.2, St.3, and St.4 can be attributed to the high concentrations of NO_2^-, NH_3, and TDS at the respective stations. PCA also showed positive correlations of *C. placentula* with NO_3^- and SiO_2 but negative correlations with PO_4^{3-} and pH. Previous studies have reported that nutrient concentration and pH play important roles in the morphological structure and pore-hole size distribution of *C. placentula* [62,63]. The ratio of silica composition was 30.71% in *Coscinodiscus* spp. [64], which may explain the negative correlation between *Coscinodiscus* spp. and SiO_2 at St.6 (Figure 6).

5. Conclusions

High spatial variations in epipelic distribution were observed along the study stations. SST, TSS, TDS, NO_2^-, NH_3, OM, and SiO_2, were considered as the most influential physicochemical variables on epipelic diatom diversity, abundance, and distribution in the study area. *Coconeis placentula*, *D. crabro*, *E. laevis*, *Actinoptychus* sp., *Amphora* sp., and *Coscinodiscus* sp. were the most abundant taxa in the study area. They showed strong correlations with SST, TSS, SiO_2, OM, NH_3, NO_2^-, pH, Chl-a, and TDS. This study was the first to describe the epipelic diatom diversity and distribution in Malaysian coastal waters, which may serve as a baseline for more studies on epipelic diatom dynamics in the future.

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