Phytoplankton composition of the mangrove ecosystem in Khanh Hoa province, Vietnam

Nguyen Thi Lan, V F Kovyazin and Phan Trong Huan

1Department of Ecology, Coastal Branch, Vietnam-Russia Tropical Centre, 30 Nguyen Thien Thuat, Nha Trang, 57100, Khanh Hoa, Vietnam
2Department of Engineering Geodesy, Saint-Petersburg Mining University, 2, 21 line of Vasilyevsky Island, St. Petersburg 199106, Russian Federation
*Corresponding email: tronghuan1369@yahoo.com

Abstract. The objective of this study was to clarify the structure of phytoplankton in the natural mangroves of Nha Phu lagoon in Khanh Hoa province, Vietnam, and their correlation with other environmental factors. The outcome of the surveys in the area showed that there was a high diversity of phytoplankton species with about 139 ones, particularly Silic algae dominance. The biomass of phytoplankton here is not high, ranging from 25 260 to 58686 cells/liter. The environmental parameters show that the area has a favorable environment for the growth of phytoplankton. The salinity of water was from 18 ‰ to 35 ‰, the temperature was between 29.4 °C and 30.1 °C, and the pH index varied from 7.03 to 7.98. Analysis of the relationship between the abundance of phytoplankton species and environmental factors pointed out that the number of phytoplankton species has an inverse linear proportion to the content of Chlorophyll-a but a direct one to the salinity and the total content of Phosphorus in seawater. Also, the biomass of phytoplankton has an inverse linear proportion to salinity and pH but follows the opposite one to the environmental temperature and the content of Chlorophyll-a.

1. Introduction
Mangroves are among the most productive ecosystems on earth, growing in coastal and estuarine regions of the tropics and subtropics [1, 2]. The biological productivity of a mangrove ecosystem is determined by its flora including mangroves and phytoplankton. The distribution and development of phytoplankton were strongly influenced by environmental factors such as light, temperature, salinity, and the content of nutritional salts [3]. The factors can cause an explosion in algae density of algal blooms, red tides [4-6].
Phytoplankton are the main foods of larvae and even adults of mollusks, crustaceans, fish [7-9]. Therefore, mangroves are habitats for larvae of many aquatic species seasonally spawning and rearing not only in the coastal areas but also offshore ones; the distribution, species composition, and density of phytoplankton may affect the distribution of fauna in mangroves, and determine most of the primary productivity of the entire open seas and oceans. According to the statistics from Indonesia [10], Malaysia [11, 12], and the Philippines [13], nearshore fishery production had a directly linear correlation with mangrove areas. Annually, mangrove-related fisheries, crustaceans, and mollusks contribute 21% (about 1.4 million tons) of the production of inshore fishery in the ASEAN region [14] which is included by
about 1.09 million tons of mangrove-related fish as well as approximately 0.4 million tons of mangrove-related shrimp, worth $1.4 billion. The area of mangroves in Vietnam decreased significantly during merely 60 years from $450 \times 10^3$ hectares in 1943 to $155 \times 10^3$ hectares in 2000 due to the lack of awareness of the importance of mangroves [15]. The causes of the decline in the country included the effect of the use of herbicides (Agent Orange) during the Vietnam War by the United States, the convertibility of mangroves into croplands, and the urbanization of cities. However, recognizing the important role of mangroves in biodiversity conservation and coastal protection, especially in mitigating the impacts of climate change, the bad practices have been stringently restricted, and the Vietnamese government has taken many measures to restore mangrove lands since 2000. In more detail, from 2000 to 2017, the area of mangroves increased from $155 \times 10^3$ hectares to $164 \times 10^3$ hectares which accounted for about 1.5% of the total forest area of the country due to artificial afforestation [16]. The Forestry Law of Vietnam classifies forests into three main categories according to management purposes: special-use forests (national parks and nature reserves), protection forests (watershed protection, coastal protection, and environmental protection), and production forests [17]. Out of the total area of mangroves, the protection mangrove forests covered the largest area, with $106414$ hectares (accounting for 73% of the total mangrove area of the country). Production mangrove ones accounted for about 18%, and special-use ones covered only 9%. The total area of mangroves in Khanh Hoa province is about 60 hectares with mainly protection mangrove forests [16]. Most of this area is artificially planted Rhizophora and Avicennia forests but only a negligible part is the natural forest located in remote areas which less affected by residential activities. This study was carried out in the natural forest area of Nha Phu lagoon in order to evaluate the physicochemical properties of the water there, the structure of the phytoplankton biome, and the relationship between these physicochemical characteristics with phytoplankton biome structure.

2. Methods and Materials

2.1. Area of research
Nha Phu lagoon in Ninh Hoa town is located in the north of Nha Trang city. This is one of the areas where aquaculture developed. Nha Phu lagoon covers an area of 4500 hectares [18]. On the East coast of NhaPhu Bay, there is a natural mangrove forest along HoaLan stream banks opposite Thi island. The dominant species of mangroves are *Rhizophora apiculata*, *Rhizophora mucronata*, *Avicennia officinalis*, and *Sonneratia alba* [19].

All ten water samples were collected at low tide in Hoa Lan stream and along the coast upstream. The depth of the stream bed is about 1.2 meters, except for point C.7 with a depth of between 0.2 and 0.5 meters and a sandy bottom.

2.2. Sampling protocols
Salinity, temperature, and pH index were measured directly on location by Hanna HI98194.

The chlorophyll-a content was analyzed according to the method of Strickland and Parsons which followed the procedure [20]: a volume of 500 ml seawater was filtered through a 0.7 μm membrane filter (Whatman GF/F filter paper), then frozen at -200°C until conducting the analysis. After filtration, the sample was extracted with 90% acetone for 2 hours and measured on a UV-Visible spectrophotometer at a wavelength of 480, 630, 647, 664, 665, 750 nm.

Seawater collected for analysis of the nutrient (total N, total P) was immediately placed in a cooler and sent to the laboratory to be tested for total nitrogen and phosphorus.

The total nitrogen concentration was determined by oxidizing nitrogen-containing compounds with potassium persulphate heated in alkali. At that time, organic and inorganic compounds containing nitrogen
were converted to nitrate. Then, the nitrate was reduced by a cadmium reducing agent to nitrite, and after this, the substance was determined by the color reaction of the Griss reagent according to the photometric method [21].

A total of phosphorus was determined by oxidation of its soluble orthophosphate compounds, followed by analysis by the Morphy and Riley method [22].

Qualifying template: Qualitative samples used in the analysis of phytoplankton species composition were collected by pyramid mesh with a mouth diameter of 30 cm and a mesh coil of 25 μm, pulled several times from near the bottom to the surface. Collected samples were placed in dark plastic vials and immediately fixed with formaldehyde solution (final concentration 5%), stored in dark and cool conditions until laboratory analysis. The phytoplankton qualitative samples were analyzed by the morphological method under the microscope. Nomenclature and taxonomy of phytoplankton were based on the data by Guiry [23].

Quantitative sample: Quantitative samples used for determining the density of phytoplankton cells were collected using a Niskin bottle of 1000 milliliters [24]. The water sample was fixed right after collection with neutral Lugol solution and wrapped in a black plastic bag and cooled until analysis in the laboratory. In the lab, 1000 milliliters of water sample was initially allowed to deposit and gradually removed water through several stages; each depositing stage lasts from 24 to 48 hours in cylindrical sedimentation tubes with a volume of 1000 milliliters, 500 milliliters, 250 milliliters, and 100 milliliters respectively. Finally, the sample was placed in a counting chamber Sedgewick-Rafter with a volume of 1 000 μl in order to count the number of cells.

2.3. Analyzing data
The obtained results of the study were processed using Excel Microsoft Office software, Statistics SPSS, and Primer 6.0. To analyze the correlation between species composition of phytoplankton, their density, and environmental parameters, Pearson’s correlation coefficient in SPSS software was applied. Comparison of phytoplankton species composition among sampling points was conducted according to similarity index in Primer 6.0 software.

3. Results and Discussion
Salinity gradually decreased from 35 ppm (when the estuary area empties into the sea downstream) to 18 ppm in upstream (table 1). The salinity of water decreased sharply from 30 ppm at station C.7 to 23 ppm at station C.8 and 20 ppm at station C.9. The surface temperature ranged from 29.4°C to 30.1°C. At the research site, the water is slightly alkaline; in the upstream area, the pH of the water was 7.02, and in the downstream area, where the stream flows into the sea, the pH of the salt reached 7.80.

According to Boyd’s conclusion [25] that the optimal pH for the growth of phytoplankton is from 6.8 to 8.0, the pH of the water from the sampling site was conducive to algae growth. The chlorophyll-a content in water samples varied from 0.660 to 3.400. The content of total phosphorus (40.5-64.5 μg/L) and nitrogen (326-716 μg/L) in water was not high.

In the study area, a total of 139 species of phytoplankton were found, which belong to 7 phyla (Bacillariophyta, Miozoa, Cyanobacteria, Cercozoa, Ochrophyta, Charophyta, Chlorophyta) (figure 1), 11 classes, 57 families, and 77 genera. Specifically, there were 99 species of Bacillariophyta algae, 26 species of Miozoa, six species of Cyanobacteria, one species of Cercozoa, two species of Ochrophyta, four species of Charophyta, and one species of Chlorophyta (table 2).
Table 1. Hydrographic parameters of the environment.

| Sampling site | Chl a (μg/l) | Total phosphorus (μg/l) | Total nitrogen (μg/l) | Salinity (ppm) | Temperature (°C) | pH |
|---------------|--------------|-------------------------|----------------------|----------------|------------------|----|
| C.1           | 0.660        | 42.6                    | 460                  | 35             | 29.5             | 7.80 |
| C.2           | 0.840        | 40.5                    | 326                  | 35             | 29.4             | 7.75 |
| C.3           | 0.850        | 49.6                    | 530                  | 35             | 29.5             | 7.92 |
| C.4           | 1.010        | 52.5                    | 662                  | 34             | 29.4             | 7.98 |
| C.5           | 1.250        | 57.6                    | 687                  | 32             | 29.4             | 7.96 |
| C.6           | 1.560        | 56.6                    | 716                  | 31             | 29.4             | 7.92 |
| C.7           | 2.080        | 55.7                    | 691                  | 30             | 29.4             | 7.49 |
| C.8           | 1.805        | 46.9                    | 695                  | 23             | 29.4             | 7.40 |
| C.9           | 3.400        | 64.5                    | 685                  | 20             | 30.1             | 7.03 |
| C.10          | 1.180        | 58.5                    | 610                  | 18             | 30               | 7.02 |

Figure 1. Correlation of phytoplankton types at water sampling sites.

Table 2. Species composition of phytoplankton in the mangrove forest of Nha Phu Bay.

| №  | Phylum          | Class | Family | Genus | Species |
|----|-----------------|-------|--------|-------|---------|
| 1  | Bacillariophyta | 3     | 34     | 54    | 99      |
| 2  | Miozoa          | 2     | 10     | 10    | 26      |
| 3  | Cyanobacteria   | 1     | 5      | 5     | 6       |
| 4  | Cercozoa        | 1     | 1      | 1     | 1       |
| 5  | Ochrophyta      | 2     | 2      | 2     | 2       |
| 6  | Charophyta      | 1     | 4      | 4     | 4       |
| 7  | Chlorophyta     | 1     | 1      | 1     | 1       |
|    | Total           | 11    | 57     | 77    | 139     |
Comparing the diversity of phytoplankton species in the mangrove ecosystem of NhaPhu lagoon with other areas such as in the Philippines, Bangladesh, Cameroon, and Indonesia, it can be pointed out that the mangrove ecosystem of Nha Phu lagoon has the highest diversity (table 3).

Table 3. Number of phytoplankton species in various mangrove forests of the world.

| №   | Research site                      | Number of phytoplankton species |
|-----|-----------------------------------|---------------------------------|
| 1   | Mangrove forests in Nha Phu, Viet Nam | 139                             |
| 2   | Mangrove estuary, Philippines [26] | 61                              |
| 3   | Mangrove forests in Sundarbans, Bangladesh [27] | 134                             |
| 4   | Mangrove forests in Cameroon [28] | 127                             |
| 5   | Mangrove forests in Perancak, Indonesia [29] | 6                               |

In the past, Nguyen Ngoc Lam [30] and colleagues studied the phytoplankton species of NhaPhu lagoon, specifically in shrimp ponds, mussels and oyster farming areas, and the lagoon's estuary waters affected directly by the offshore water. The outcomes of the Nguyen Ngoc Lam’s research revealed that 229 phytoplankton species were recorded, of which Silica algae accounted for 150 species. It was noteworthy that there were 26 species of toxic algae found mainly in aquaculture areas. Comparing the results of this study with the results of Nguyen Ngoc Lam and his colleagues, the number of phytoplankton species in mangroves is fewer, the number of toxic algae species in mangrove ecosystems was significantly fewer. There were only 5 species of toxic algae including Pseudo-nitzschia spp., Dinophysis caudata, Diesthysis miles, Noctiluca scintillans, and Trichodesmium erythraeum. The large difference in the number of such toxic algae was explained by the different locations of water samples with different levels of influence of anthropogenic activities, the content of polluting substances.

Regarding species composition, both studies showed the predominance of Silic algae species. In the study of Nguyen Ngoc Lam, the number of diatoms was 150 species out of a total of 229 phytoplankton species recorded in the whole NhaPhu lagoon. In the mangrove ecosystem of Hoa Lan stream, there were 99 species of silicon algae out of a total of 139 species recorded. The number of Silic algae species at each sampling site ranged from 39 to 71 species (figure 1), accounting for 69.23% to 87.30% of the total phytoplankton species.

The comparison of phytoplankton species composition between the sampling sites was made according to the similarity index and presented in a matrix form (table 4). The above calculations showed that there was a similarity in composition at sampling points. This was explained by the influence of tides leading to the water exchange between upstream and downstream areas, which encouraged phytoplankton species to travel along with the water flow and mix species composition among the areas.

Table 4. Index of similarity of species composition between sampling site.

| C.1 | C.2 | C.3 | C.4 | C.5 | C.6 | C.7 | C.8 | C.9 |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| C.2 | 75.71 |
| C.3 | 75.00 | 67.40 |
| C.4 | 72.48 | 70.13 | 67.97 |
| C.5 | 68.06 | 57.72 | 74.32 | 72.73 |
| C.6 | 63.70 | 55.71 | 64.75 | 71.43 | 76.64 |
| C.7 | 60.15 | 49.28 | 61.31 | 58.18 | 76.19 | 70.83 |
| C.8 | 76.92 | 69.57 | 72.50 | 73.68 | 76.56 | 73.95 | 63.25 |
| C.9 | 60.14 | 52.70 | 66.67 | 66.67 | 78.26 | 77.36 | 78.85 | 70.87 |
| C.10| 67.11 | 58.60 | 73.08 | 66.67 | 82.26 | 74.78 | 72.57 | 73.53 | 81.30 |
Tides also affected the density of phytoplankton cells. The density ranged sharply from 25260 cells per liter to 58.686 cells per liter. The density upstream area was higher than in the downstream one.

Applying Pearson's correlation coefficient, the result of the analysis of the correlation between species composition of phytoplankton, their density, and environmental parameters (table 5) represented two following points.

There was no linear correlation between the number of species and the phytoplankton density. The number of phytoplankton species had a directly linear correlation with water salinity ($r^2 = 0.685$, sig. = 0.029) and the total of phosphorus content in water ($r^2 = 0.788$, sig = 0.007) and an inversely linear correlation with chlorophyll-a content ($r^2 = -0.690$, sig = 0.027).

The density of phytoplankton cells had a directly linear correlation with temperature ($r^2 = 0.772$, sig = 0.009) and chlorophyll-a content ($r^2 = 0.705$, sig = 0.023) and had an inversely linear correlation with salinity ($r^2 = 0.705$, sig = 0.023). $r^2 = -0.847$, sig = 0.002) and pH ($r^2 = -0.852$, sig = 0.002).

This could be explained by the fact that the samples were taken at low tide when the water from upstream flowed into the sea and brought phytoplankton to the downstream, which caused the accumulation of them in some areas. In addition, under the influence of the increase in temperature and light energy, the physiological activity of algae increased, thereby rising their density and increasing the content of chlorophyll-a. These physiological processes increased the acidity but decreased the pH of the water [31]. The inverse correlation between phytoplankton density and water salinity could be explained by the fact that the samples were taken downstream where there was a strong exchange of water between the stream and the sea making algae density dispersed.

**Table 5. Coefficient of correlation between the species composition of phytoplankton, its density and environmental parameters.**

| Environmental parameters | Number of phytoplankton species | Density of phytoplankton |
|--------------------------|---------------------------------|--------------------------|
| Salinity                 | 0.685                           | -0.781                   |
| Temperatures             | -                               | 0.772                    |
| pH                       | -                               | -0.852                   |
| Chlorophyll-a            | -0.690                          | 0.705                    |
| Totalphosphorus          | 0.788                           | -                        |
| Totalnitrogen            | -                               | -                        |

The analysis of the relationship between the abundance of phytoplankton species and environmental factors showed that the number of phytoplankton species had an inversely linear correlation with the chlorophyll-a content and a directly linear proportion to the salinity and the total of phosphorus content in seawater. The biomass of phytoplankton was inversely correlated with salinity and pH and directly with temperature and chlorophyll-a content.

**4. Conclusion**

The study identified 139 species of phytoplankton belonging to 7, 11 classes, 57 families, and 77 genera. Of the species, diatoms predominated, accounting for between 69% and 87% of the total species. In terms of the phytoplankton species, there were 99 species of Bacillariophyta, 26 species of Miozoa, 6 species of Cyanobacteria, 4 species of Charophyta, 2 species of Ochrophyta, 1 species of Cercozoa, and 1 species of Chlorophyta. The species diversity of phytoplankton in the mangrove ecosystem of the sampling site exceeded the number of algae inhabiting mangrove forests in other countries.

It was found that the species composition was determined by the concentration of Chlorophyll-a (an inversely linear correlation) and salinity, the total of phosphorus content (a directly linear correlation); and phytoplankton density directly correlated with temperature and Chl content and negatively correlated with salinity and pH.
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