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Original Article

Environmental Factors Influencing Chlorophyll-a Concentration in Tri An Reservoir, Vietnam

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Abstract: Chlorophyll-a (Chl-a) has been used extensively as an essential indicator of trophic state in the assessment and monitoring of surface water quality environments. The environmental factors can influence Chl-a concentrations; thus, to determine the relationship between Chl-a concentration and factors. The research was carried out in dry season (March 2016) and wet season (September 2016) in Tri An reservoir, Dong Nai Province, Vietnam and performed by Spearman's correlation analysis and Linear regression analysis. The result showed that Chl-a varied between 12.84 and 783.51 µg/L and was quite different a cross stations in two surveys. Factor analysis and the best models revealed the association of strong physico-chemical with Chl-a concentration. The Chl-a was significantly positively correlated with Total Suspended Solids (TSS) and negative with Nitrate (NO_3^-) in the dry season, while in the wet season the positive relationships between Chl-a concentration and Dissolved Oxygen (DO), Temperature and a strong negatively correlated with Phosphate (PO_4^{3-}) correlation were found. This relationships inferred that the nutrients brought by the influx of reservoir into the study area have contributed to control the growth and abundance of phytoplankton. Thus, the importance of environmental factors in structuring Chl-a concentration may be used to guide the conservation of the aquatic ecosystems in the reservoir.

Keywords: Chlorophyll-a concentration, environmental factors, Linear regression analysis, Spearman's correlation analysis.

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1. Introduction

In the aquatic ecosystem, phytoplankton plays a vital role in nutrient cycling and the overall food web [1]. Chlorophyll-a (Chl-a) concentration is widely recognized as a proxy for phytoplankton biomass and used in eutrophication studies worldwide [2]. Moreover, Chl-a is an essential indicator of water quality [3, 4] and the method for obtaining its concentration is more straightforward and faster than the classical analysis of phytoplankton biomass [5]. Thus, Chl-a has been widely used in the assessment and monitoring of aquatic environments [4].

The spatial-temporal variability of phytoplankton, so Chl-a depends primarily on physical factors together with the availability of nutrients [1]. Chl-a concentrations may be influenced by many environmental factors and have been studied in recent researches. Chl-a concentration can be affected by nutrient availability (primarily nitrogen and phosphorus concentrations) and water transparency [2, 4]. The study in 21 reservoirs of central Brazil indicated a positive relationship with total phosphorus concentration and depth. Besides, turbidity was negatively correlated with Chl-a concentration [2]. Besides, the significant factors influencing Chl-a concentrations were total phosphorus (TP) and water velocity (U) in three river-connected lakes (Dongting Lake, Poyang Lake, and Shijiu Lake) of the Yangtze flood-plain in 2004. Moreover, multiple relationships, including total nitrogen ($\log_{10}TN$) and water depth ($\log_{10}Z$) were established [6]. Light availability and nutrients were recognized as limiting factors to phytoplankton growth [7].

Tri An Reservoir is located in Dinh Quan District, Dong Nai Province, Vietnam. Built-in 1986, Tri An Reservoir is a multipurpose reservoir and used for multiple purposes such as hydroelectric power, flood control, domestic and industrial water supply, fisheries and irrigation. However, fish caging, wastewater from the factories and human activities has led to nutrient enrichment of the reservoir, supporting phytoplankton growth in general and green algae development in particular [8]; therefore, it may affect Chl-a concentration.

The study aimed to determine which Physico-chemical parameters influence the distribution of Chl-a concentrations; therefore, contributing to useful in the assessment and monitoring of aquatic environments.

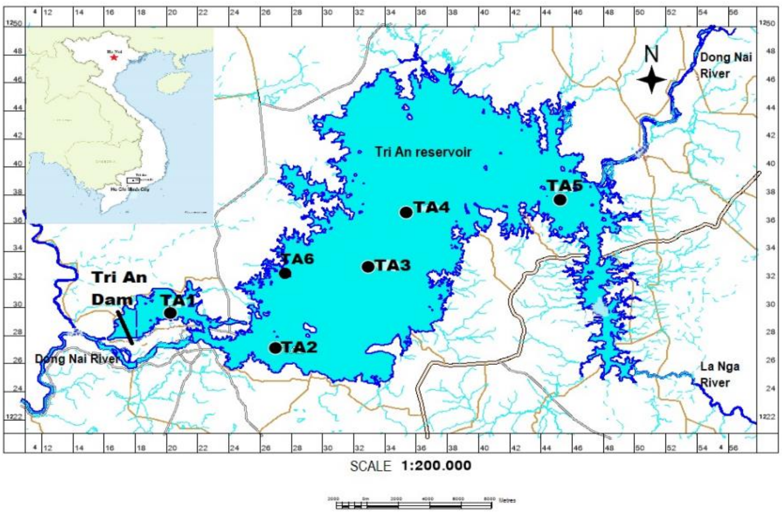

Figure 1. Map of Tri An Reservoir and the 6 sampling sites.

2. Materials and Methods

2.1. Study Area

Samples were taken at 6 sites which symbols were from TA1 to TA6 in the reservoir with distinct characteristics: sub-reservoir area (TA1), main reservoir area (TA2–TA6) with TA5, and TA6 were represented areas of concentrated fish caging (Table 1 and Figure 1).

Table 1. Locations of sample sites

Sites	Sampling coordinates	
	Latitude	Longitude
TA1	11°07'30.5"N	107°01'01.7"E
TA2	11°06'25.0"N	107°05'06.9"E
TA3	11°08'57.8"N	107°07'49.7"E
TA4	11°11'20.9"N	107°09'32.3"E
TA5	11°10'47.2"N	107°16'10.6"E
TA6	11°08'18.4"N	107°04'11.9"E

2.2. Field Sampling

Two surveys were conducted at 6 sites in Tri An Reservoir in March 2016, representing the dry season and September 2016 represents the wet season. The parameters such as water temperature, pH, Dissolved Oxygen (DO), and Secchi disk were collected from the surface and measured *in situ* by using a multi-parameter (Hach 156, Co, USA). Measuring inorganic nutrient parameters, the surface water sample was collected by using plastic container 2 liters, then kept in the icebox and transferred to the laboratory for physicochemical analysis.

Planktonic diatom samples were collected from the surface waters by towing a conical net made of bolting silk with 25 µm mesh size. Subsequently, samples were kept in 150 ml plastic bottle, preserved in 4% neutralized formalin and used for qualitative analysis, and Chl-a analysis samples which collected by using plastic cans (each with a capacity of 2 liters) of surface waters, then reserved in 4% neutralized formalin for qualitative samples.

2.3. Chemical and Chlorophyll-a Analysis and Planktonic Diatom Identification in the Laboratory

Chemical parameters (TSS and nutrients) were measured according to APHA methods [9] in which samples for dissolved nutrients such as nitrate (N-NO_3^-), phosphate (P-PO_4^{3-}), total nitrogen (TN), and total phosphorus (TP) were analyzed colorimetrically in triplicate with a spectrophotometer (Hach DR/2010).

In order to analyze Chl-a concentration, about 50-300 mL samples were filtered through filter paper GF/C. The filter was subsequently frozen until sample processing. Chl-a was dissociated with 90% acetone solution overnight at room temperature and without light. Then the sample was centrifuged at 400 rpm, 20 minutes to discard scum. Chl-a in extract solution was analyzed by UV-DR-500 spectrophotometer (Hach, USA).

Samples were examined with an Olympus BX51 light microscope, equipped with differential interference contrast at a magnification of ×40. Identification was based on morphology following some research books [10, 11]. The classification of green algae into taxonomic groups was followed by AlgaeBase web [12]. Quantitative samples were deposited in measuring cylinder in 48 hours, then exsiccated to about 5 mL. At least 400 cells were counted under Sedgewick counting technique by the method of Sournia (1978) [13].

2.4. Data Analysis

Statistical calculations were performed using Statgraphic centurion XV. One-way analysis of variance (ANOVA) was used to test the differences among the groups of study sites and two seasons. The analysis was completed, then using Tukey's HSD test the significant difference. The correlation between Chl-a concentration and environmental parameters was determined by Spearman's correlation analysis method, and then linear regression analysis was performed. All variables were log-transformed $(\log + 1)$ to normalize their distributions before analysis.

3. Results

3.1. Physico-Chemical and Nutrient Variables

The average Physico-chemical variables concentrations from the surface waters of Tri An Reservoir in both dry and wet seasons were shown in Figure 2. The pH varied between 6.76-8.07 with a minimum rate in dry season and maximum rates occurring in the wet season. The normal distribution for pH was similar in both seasons. The surface water temperature varied between a minimum rate of 29.38 ℃ in the dry season and a maximum rate of 30.04 ℃ in the wet season. The temperature of the survey in Tri An reservoir was relatively stable and not too large fluctuations between the samples sites and between the two surveys. The DO values ranged from 4.50 to 7.03 mg/L with the minimum occurring in the wet season and the maximum in the dry season. The transparency of the water was a factor that apparently played a role in the concentration of the phytoplankton [14]. Water transparency was assessed through the Secchi disk index. The Secchi disk varied between 20.28 and 164.9 cm, with its minimum rate belonging to the wet and maximum rate to the dry season. NO_3^- varied between 0.25 and 0.75 mg/L with the minimum occurring in the dry season and the maximum in the wet season. PO_4^{3-} ranged between 0.06-0.122 mg/L with its minimum during dry and maximum during wet season. In the wet season, station TA5 had a value of PO_4^{3-} higher than the other stations. TN varied from 2.47-5.09 mg/L with both minimum and maximum rates occurring in the wet season. TP varied between a minimum rate of 0.15 mg/L in the dry season and a maximum rate of 0.38 mg/L in the wet one. TP was measured in two surveys to indicate that the wet season was higher than in the dry season. The concentration of TSS was another commonly used indicator for water quality assessment. TSS varied between 12.57 and 226.21 mg/L with its minimum and maximum rate during dry season. TSS values between the wet season and the dry season differed from survey sites. In the stations such as TA3, TA4, TA5, TA6, TSS values were higher in the wet season than in the dry season and gradually decrease from TA3 to TA6. Moreover, according to the classification of eutrophication level of Håkanson et al. (2007) [15], the results of Secchi dish, TN and TP analysis in Tri An reservoir have been classified into Eutrophic to Hypertrophic.

The results of One-way ANOVA, and Tukey's HSD test showed that the mean of pH, temperature, and TN have not been significantly different ($p>0.05$), while the significant difference in other environmental variables have been detected between both seasons ($p<0.05$). In general, the mean of DO and Secchi dishes in the dry season have been significantly higher than those of the wet season ($p<0.05$). In addition, DO and Secchi dish values in stations TA5 and TA6 were always lower than other stations in both seasons; thus affecting fish caging in this area. On the other hand, the other environmental variables such as NO_3^-, PO_4^{3-} and TP in the wet season were higher than in the dry season in most of the survey sites.

3.2. Chlorophyll-a Concentration

Chl-a varied between 12.84 and 783.51 µg/L with the minimum occurring in the wet and the maximum one in the dry season (Figure 3). The concentration of Chl-a measured in Tri An reservoir in two seasons was quite different at stations: TA2, TA3, TA4, TA5, TA6 (ANOVA, $p<0.05$). The dry season had the highest Chl-a content at station TA2 and gradually decreases to station TA6, while in the wet season, the highest Chl-a content was TA3 station and the lowest was TA5 station. Besides, the TA5 station was always lower than other stations in both survey seasons. These results showed that abundance of phytoplankton in TA5 station was very low. Besides, One-way ANOVA and Tukey's HSD test showed that the mean of Chl-a in TA1 station in the two seasons was not significant ($p>0.05$).

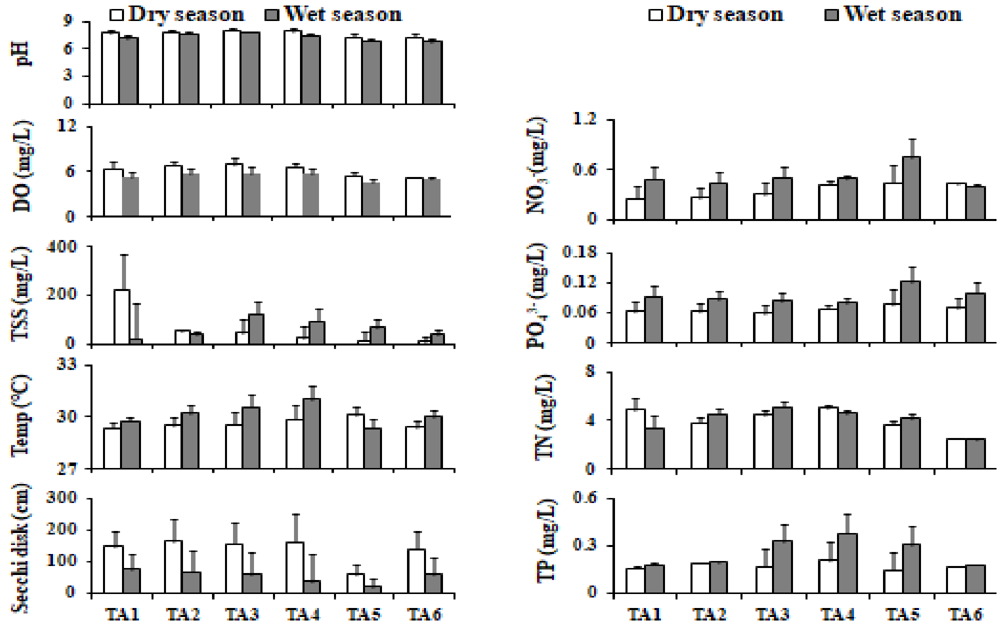

Figure 2. Median (mean ±SD) water quality variables from sampling sites in dry and wet seasons

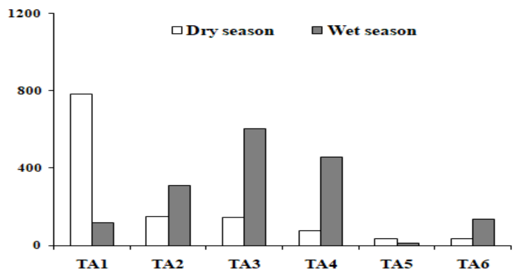

Figure 3. Chlorophyll-a concentration in the two seasons in Tri An Reservoir.

3.3. Green Algae Community Structure and Abundance

In this study, the community structure and abundance of phytoplankton were shown by studying the community structure and abundance of green algae. A total of 77 green algae species, belonging to 26 genera, have been identified during the study period and the generas such as *Dictyosphaerium*, *Scenedesmus*, *Sphaerocystis*, and *Staurastrum* were the dominant ones.

Abundance varied from 6.33×10^4 cells/L to 1.00×10^6 cells/L with the highest from TA3 station and the lowest from TA6 station were observed in wet season (Figure 4). During the wet season, the frequent occurrence of colonial green algae such as *Dictyosphaerium* sp., *Oocystis* sp.,... have contributed to a significant increase in abundance of green algae. In contrast, *Scenedesmus* sp., *Staurastrum* sp. and *Cosmarium* sp. species appeared more frequently in the dry season. In dry season, abundance was high at stations TA3 and TA4, while in the wet season, abundance of stations TA1, TA2 and TA6 were higher than the others. The TA5 station had relatively lower abundance than the others in both survey seasons. In wet season, green algae and nutrients, organic compound from upstream and on both sides of the reservoir were led to enter to the downstream which may result the density increase especially the stations TA1, TA2, and TA6. At the concentrated fish caging, TA6 in wet season had a very high abundance, while the density of TA5 station was relatively low in both seasons. As TA5 station is the upstream area where the current is strong and will sweep green algae down the downstream.

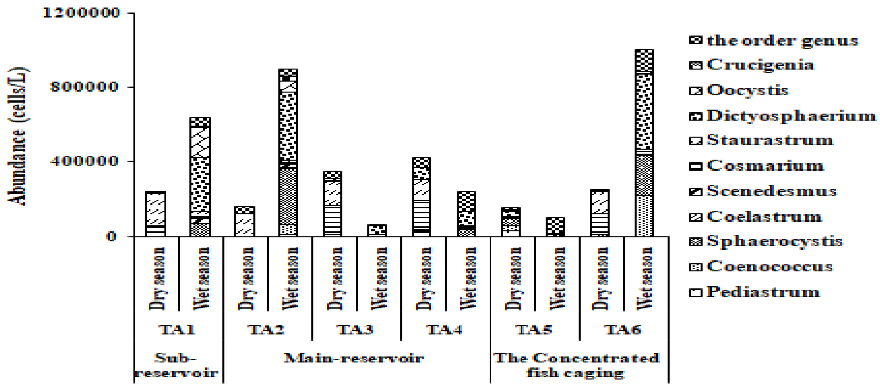

Figure 4. Green algae abundance in the dry and wet season.

3.4. Relation of Environmental Factors to Chlorophyll-a Concentration

Table 2. The correlation coefficient between Chl-a and environmental variables in the dry season

The environmental variables	Chl-a concentration (Correlation Coefficient)
DO	0.1797
Secchi disk	0.2774
TSS	0.0476*
Temperature	0.1797
NO_3^-	0.0350*
PO_4^{3-}	0.0445*
TN	0.1797
TP	0.7954
pH	0.2248

Note: * Correlation is significant at the 0.05 level.

The correlation between Chl-a concentration and environmental variables were shown by Spearman's correlation analysis and linear regression analysis.

The results of the Spearman's correlation analysis between the environmental variables and Chl-a concentration in the dry season were shown in Table 2.

Chl-a concentration was correlated with TSS, NO_3^- and PO_4^{3-} (r=0.8857, r=-0.9429, r=-0.8986, p<0.05, respectively), while the other variables did not correlate with Chl-a concentration (p>0.05) such as pH, DO, Secchi disk, TN and TP. Then, the linear regression analysis which indicated the relationship between Chl-a concentration and environmental variables (only TSS and NO_3^-) were demonstrated in Figure 5 that Chl-a had a strong positive correlation with TSS and were able to obtain a model with a relatively high predictive power (adjusted R^2= 99.4947%, p<0.001), while Chl-a was negatively correlated with NO_3^- and a substantially higher predictive capability (R^2=78.0102%, p= 0.0124).

The results of the Spearman's correlation analysis between the environmental variables and Chl-a concentration in the wet season were indicated in Table 3.

Table 3. The correlation coefficient between Chl-a and environmental variables in wet season

The environmental variables	Chl-a concentration (Correlation Coefficient)
DO	0.0022*
Secchi disk	0.1680
TSS	0.5808
Temperature	0.0191*
NO_3^-	0.0899
PO_4^{3-}	0.0008*
TN	0.5398
TP	0.7779
pH	0.0687

Note: * Correlation is significant at the 0.05 level.

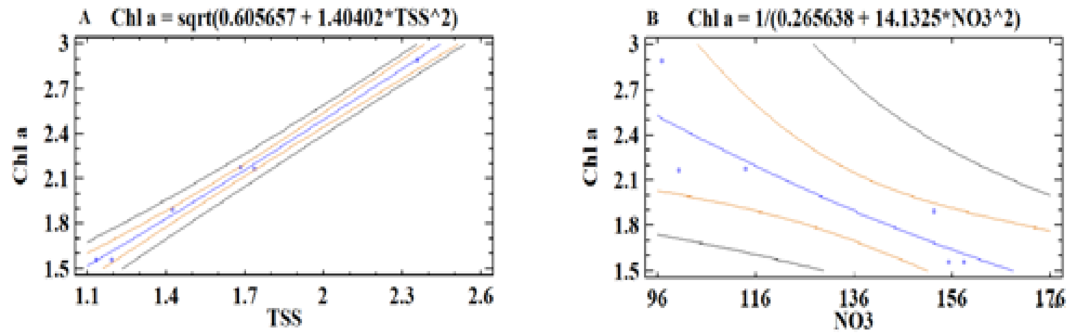

Figure 5. Model of Linear regression analysis: A. The correlation between Chl-a concentration and TSS; B. The correlation between Chl-a concentration and NO_3^-.

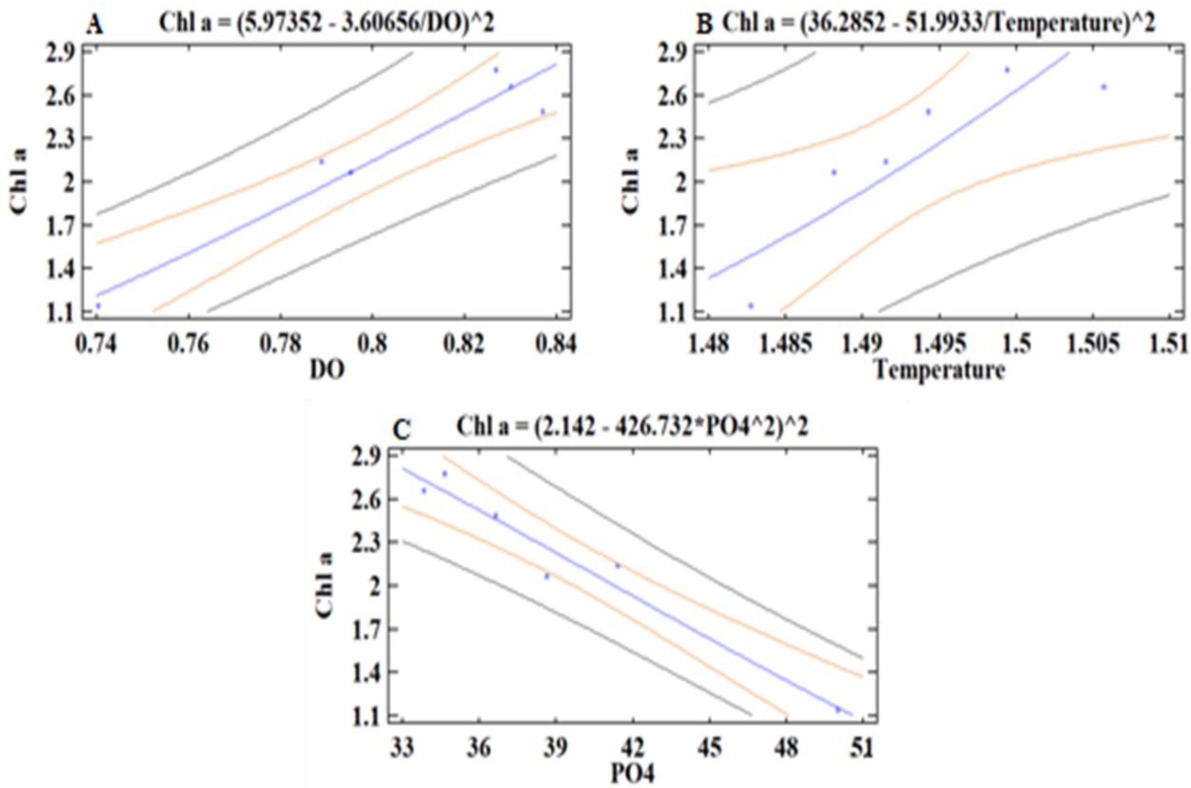

Figure 6. Model of Linear regression analysis: A. The correlation between Chl-a concentration and DO; B. The correlation between Chl-a concentration and Temperature; C. The correlation between Chl-a concentration and PO_4^{3-}.

In the wet season, Chl-a concentration was correlated with DO, PO_4^{3-} and Temperature (r=0.9612, r=0-0.9761, r=0.8849, $p<0.05$, respectively). pH, Secchi disk, TSS, NO_3^-, TN, and TP did not correlate with Chl-a concentration ($p>0.05$). Besides, the simple linear regression between Chl-a concentration and environmental variables resulted in a model with a substantially higher predictive capability (Figure 6) that illustrated the *significant*ly positive relationships between Chl-a concentration and DO, Temperature were found in this research with the high adjusted coefficient of simple linear regression models (R^2=91.8403%, p=0.0016%; R^2=78.9263%, p=0.0113, respectively). In addition, a simple linear regression between Chl-a and phosphate (PO_4^{3-}) resulted in a model with a strong negatively correlated (R^2= 95.053%, p=0.0006).

4. Discussions

The result of compared with seasonal changes, Chl-a was found in the wet season was higher than in the dry season in most of the survey sites exception stations TA1, TA5 which might be influenced by environmental factors. The water transparency is a factor indicating the quantity of the phytoplankton as Chl-a concentration; any areas with high water transparency value and a low nutrient distribution value result in having low concentration of the Chl-a [16]. Station TA1 and TA5 had high water transparency and TSS values and low nutrients distribution values in the wet season; thus, Chl-a was in the dry season than the wet season. Furthermore, Sanders et al. (2001) indicated that the Chl-a content changed according to season in the areas affected by nitrate and phosphate [17]. This study reported that nitrate and phosphate in wet season were higher than in dry season in most of the survey sites. Moreover, in the wet season, nutrients might be from fish caging and wastewater to lead to nutrient enrichment of La Nga river entering the reservoir, consequently the growth of the phytoplankton. As a result, Chl-a in wet seasons was higher than in dry season. Also, stations TA1, TA5 were found the highest and lowest Chl-a concentration, respectively. As TA1 station is a downstream area, its depth and width are small, so the amount of phytoplankton from upstream areas will concentrate on here, while TA5 station is upstream area where the current is strong, the phytoplankton will be swept away by the flow. Besides, according to the classification of water eutrophication level of Håkanson et al. (2007) with the Chl-a concentrations, Tri An reservoir is classified into Eutrophic to Hypertrophic [15].

In the dry season, Chl-a concentration had a significant positive correlation with TSS but was negatively correlated with NO_3^-. That means when TSS increased Chl-a concentration increased and when there was an increasing NO_3^- content the Chl-a concentration decreased. Surface water quality is also assessed through the measured TSS. TSS consists of organic and inorganic materials suspended in the water though bacteria and algae can also contribute to the total solids concentration [18]. When increase TSS, the light transmission through the water will be decrease so resulting in a decrease of phytoplankton primary production [19]. Thus, this result was in line with recent studies indicating that Chl-a and TSS relationships tend to be positive. For instance, the linear regression was indicated the relationship between Chl-a concentration and TSS which shown that TSS affected the distribution of Chl-a concentration on both wet and dry months in the study area of Cirebon marine waters. These equations also showed that TSS had positive and direct linear relationship with Chl-a in both seasons [20]. As an essential nutrient, nitrogen is a limiting factor for phytoplankton growth and NO_3^- is the most commonly available form of nitrogen [21]. Previous researches also reported that the relationship between Chl-a concentration and NO_3^- was described. The research in coral reef areas around Unguja, Zanzibar, Tanzania, Chl-a concentration was temporally significantly correlated with NO_3^- but it was positive [22]. Besides, a negative relationship between NO_3^- and Chl-a was corroborated in the study in some lakes of the Upper Paraná floodplain [23]. Phytoplankton significantly affects NO_3^- and this factor is considered an essential determinant of phytoplankton biomass in lake [24]. Generally, like ammonia, nitrate is the nutrient that the phytoplankton and water plant used to synthesize protein for their growth [16, 25].

In the wet season, two environmental parameters namely DO and water surface temperature were positively correlated with Chl-a concentration. On the contrary, PO_4^{3-} had a significant negative correlation with Chl-a concentration. When DO, Temperature increased Chl-a concentration and there was an increase PO_4^{3-} content the Chl-a concentration decreases. It has had also many recent studies to show the relationship between Chl-a concentration and DO, Temperature, PO_4^{3-}. The study of relationship between DO and Chl-a concentration of Bang Pa Kong Estuary was positive that decreased with depth [26]. It was temporally significantly positively correlated with DO and PO_4^{3-} in coral reef areas around Unguja, Zanzibar, Tanzania [22]. DO is one of the most important environmental factors for phytoplankton health because of its effect in a number of biogeochemical processes such as respiration and metabolism that influence their life which related to phytoplankton biomass as determined by Chl-a concentration [27]. The increase in DO can be attributed to photosynthetic release [28]. Water temperature affects the control of the growth and distribution of the phytoplankton [16, 29]. When the water warms throughout the dry season, phytoplankton grows to higher concentrations or as there is a heavy rain more nutrients get washed into the lake to cause algae bloom and eutrophication. The studies of relationship between Chl-a and temperature have been widely studied. The temperature had negative significant correlation with Chl-a concentration in coral reef areas around Unguja, Zanzibar, Tanzania [22] while the study in the Bangpakong river reported that the Chl-a content had positive correlation to water temperature [16, 30]. Besides that, the study in Srinadharin reservoir, Kanchanaburi province, Thailand, Chl-a concentration determined to have a positive correlation to water temperature and dissolved oxygen [22]. Moreover, in this study, a strong negative correlation between Chl-a and phosphate (PO_4^{3-}) resulted. Phosphate is one of the most important factors directly influencing the growth of the phytoplankton and they are also causes of eutrophication [31]. The study around the Wachiralongkorn Reservoir reported that the quantity of the Chl-a had positive correlation to nitrate and orthophosphate [16, 32].

In general, Chl-a concentration was affected by environmental factors such as TSS, DO, temperature and nutrients (NO_3^- and PO_4^{3-}). Primary product of the water sources is the biomass of the phytoplankton be produced by the photosynthesis and Chl-a as the main factor for the photosynthesis and is able to indicate the biomass of the phytoplankton [16, 33]. The environmental factors can be changed for the growth of the phytoplankton. Nutrients are a supportive factor to grow and life of phytoplankton as nitrate and phosphate, all of which used to photosynthesis by phytoplankton and other factors. The change of the nutrients can put impact on the quantity of the Chl-a, consequently influence the primary product of the water sources. Therefore, nutrients play a crucial role in phytoplankton growth and abundance. Besides, nutrients associated with eutrophication of lakes and reservoirs which corresponded to the previous studies. Reservoir construction can lead to increased eutrophication because sediments in reservoirs can act as a source of nutrients of which the nitro and phosphor compounds can cause eutrophication [8, 34]. Moreover, the case of Tri An Reservoir in Vietnam is not an exception [8]. Thus, predictive models for relationships between Chl-a concentration and nutrients or include other factors are critical ecological tools for water quality management.

5. Conclusions

The present study aimed to determine the relation of physico-chemical parameters and Chl-a concentration in Tri An Reservoir. The concentration of Chl-a measured in Tri An reservoir in two seasons was quite different at stations and was found in the wet season are higher than in the dry season in most of the survey sites. Through the factors such as nutrients (as nitrogen and phosphorus) and

Chl-a concentration, the water quality in Tri An reservoir was found to be eutrophic state and tends to change in a negative direction. Besides, the result showed that the primary underlying factors influenced Chl-a concentration in Tri An Reservoir during the sampling period were determined from Spearman's correlation analysis and Linear regression analysis. The strong positive correlations that Chl-a concentration was effected by TSS, DO, and temperature. Besides, the significant negative association of Chl-a with nutrients in the study area which suggested that nutrients provided good environmental conditions and controlled the growth and abundance of phytoplankton.

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