

The Chlorophyll-a Response of Phytoplankton to Ratio N/P in Different Coastal Waters

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ABSTRACT

Phytoplankton growth is influenced by the presence of nutrients N (nitrogen) and P (phosphor). Each region has a specific N/P ratio, due to the influence of anthropogenic inputs. This study aimed to assess the response of phytoplankton chlorophyll-a (Chl-a) biomass due to differences in N/P ratio by the influence of river flow in the north coast of Java; Jobokuto Bay (Jepara), the coastal waters of Semarang and the front of the Cisadane river. N-nutrients were analyzed in the form of N-NO₃ (nitrate), and N-NH₄⁺ (ammonium ions), and orthophosphate ions (P-PO₄²⁻). Nutrient analysis was spectrometric, using the reduction methods (nitrate), ammonium (indophenol-blue), and phosphate (molybdenum-blue). Test for site differences using Kruskal-Wallis, followed by a posthoc test. The results showed that Semarang waters had a lower N/P (Stoichiometric) ratio than Jepara and Cisadane, which can be used to predict that P nutrient input is higher than N. This high P input impacts microalgal development (chlorophyll-a). In addition, we also found Semarang waters to have higher speciation of inorganic N in the form of ammonium, which is one of the drivers of eutrophication in these waters. The use of a ratio of N/P is very important in estimating the eutrophication process and can be used to estimate the dominance of nutrients entering the water due to anthropogenic activities in the upstream area.

Keywords: chlorophyll-a, coastal, nitrogen, phosphate, stoichiometry.

INTRODUCTION

Coastal waters are an area that receives many influences from the mainland and the sea. High human activity on land has had a noticeable impact on increasing the input of chemical elements into estuaries through river flow [Wang et al., 2015; Maslukah et al., 2019; Prayitno and Afdal, 2020]. In recent decades, anthropogenic activities have greatly increased the amount of nutrients available to coastal ecosystems worldwide [Malone and Newton, 2020; Mathew et al., 2021]. The input of nutrients into the coastal environment from terrestrial sources can have significantly different proportional changes in the ratio of Redfield (Si:

N:P = 16:16:1) [Prayitno and Afdal, 2020; Baliarsingh et al., 2021]. This can lead to variability in chlorophyll-a (Chl-a) concentrations [Malone and Newton, 2020] and alter phytoplankton species composition, a variable of ocean primary productivity. Coastal waters occupy ~8% of the world's oceans and contribute 15–30% of ocean primary production [Simpson and Sharples, 2012; Bindoff et al., 2022]. Phytoplankton, containing green pigment (Chl-a) are the most important primary producers of aquatic environments and account for up to 50% of global primary production [Field et al., 2016]. The content of Chl-a in phytoplankton is >50% and can be used as an indicator of eutrophication and depict the biomass

of phytoplankton. Eutrophication has positive impacts related to the abundance of plankton and increased fish production. Conversely, its negative impact causes the oxygen content in the water to be low and results in mass fish kill. The concentrations of Chl-a are frequently used to calculate phytoplankton biomass and water productivity for use in monitoring water quality and managing marine resources [Zhang and Han, 2015; Agirbas et al., 2017; Poddar et al., 2019].

Some of the environmental parameters in coastal waters that affect the life of phytoplankton are nutrient availability and light intensity. Wind and currents also contribute to the mixing process in such waters. The type of nutrients in a specific quantity is required for phytoplankton growth, and biomass increases, and influences species composition [Ramos et al., 2017]. Historically, nitrogen is the most common nutrient-limiting growth of phytoplankton [Dodds et al., 2016]. In the process of energy conversion during phytoplankton photosynthesis, phosphorus (P) is a crucial component. Phosphorus (P) plays a role in many metabolic reactions and is an essential element for life as it is present in biological molecules, including nucleic acids, co-enzymes, phosphoproteins, and phospholipids [Tian et al., 2021]. In addition, P is one of the main limiting elements of biomass production in ecosystems and can be the cause of ongoing eutrophication in waters [Xiong et al., 2020]. The diatoms, a type of phytoplankton that dominates coastal ecosystems, will be inhibited in their growth due to limited available P [Trommer et al., 2013; Poornima et al., 2014]. Marine ecosystems with high P nutrients utilize nitrogen (N) and carbon (C) from the atmosphere. Increases in phosphorus (P) can trigger cyanobacterial blooms, while increases in C or N in the absence of P enrichment have a slight effect [Wisha et al., 2018; Maslukah et al., 2019].

The northern area of Java is discharged by many of the rivers. Some of these rivers include the BKB (Banjir Kanal Barat) and BKT (Banjir Kanal Timur) rivers (in Semarang), Serang and Wiso rivers in Jepara, and the Cisadane river in Serang, Banten. Different levels of human activity on land will affect nutrient inputs to coastal waters. The results of Maslukah et al. [2018] showed nutrients of NO_3 and PO_4^{2-} from two rivers that have inputs into Jepara waters reached 19.66 tons/month and 2.15 tons/month and had caused the chlorophyll-a in coastal waters to reach the range of 0.55–5.21 mg/m^3 (average = 1.29 mg/

m^3). Meanwhile, in Subiyanto [2017] in the estuarine waters of the West Flood Canal (BKB), the chlorophyll-a value is quite high and the water condition is eutrophic. Several studies related to the role of nutrients N, and P and their ratios have been extensively conducted in lakes [Dodds et al., 2016; Bennet et al., 2017], but are limited in the marine ecosystems [Poornima et al., 2014]. In freshwaters, P is declared as limiting, while in the sea, N and estuary are limiting [Maslukah et al., 2019]. However, the controversy about the relative role of both nutrients is still under debate in controlling the eutrophication of both waters. Some studies have stated that N plays a significant role, whereas others have argued that restricting P inputs are adequate to manage eutrophication [Schindler, 2012; Maslukah et al., 2019].

Studies on a ratio of N/P to the concentration of chl-a in the northern coastal waters of Java are still limited in space and time. The research of Maslukah et al. [2018] in Jepara waters explained that high N nutrients must be balanced with P nutrients. This P element is significant as a limiting nutrient when Chl-a is high. In addition, it is also explained that the distribution pattern of Chl-a in Jepara follows the ion of PO_4^{2-} . However, further studies are still needed, to be able to explain the relationship between the two variables, so that their variability can be studied. In addition, the increase in anthropogenic activities in recent decades has increased significantly, so it is necessary to continuously monitor the biogeochemical properties of a coastal water area, to strengthen biogeochemical observations in coastal waters.

This study aims to investigate the role of N and P nutrients, and stoichiometry ratios on Chl-a variability in three estuaries from the North coast of Java and calculate their trophic levels. The results presented in this research are a preliminary survey of coastal waters from the north coast of Java that are quite vulnerable to anthropogenic activities.

MATERIALS AND METHODS

The three research locations were the coastal waters of Semarang, Jepara, and Banten. Semarang waters were sampled from the BKT estuary towards BKB; Jepara was Jobokuto Bay, which is influenced by Wiso River; and Banten coastal waters were taken from the Cisadane River estuary. A map of the research locations is presented in Figure 1. Samples were taken using a water



Figures 1. The study areas (a) Semarang (b) Jobokuto Bay, Jepara and c) Cisadane, Banten

sampler from ± 30 cm from the surface. Samples for analysis of P, N, and chl-a were transferred to polytene sample bottles. Before filling, the bottle was washed using the same sample water. After arriving at the laboratory, 500 ml samples were filtered. Samples not examined on the same day can be frozen and tested the following day [Maslukah et al., 2018].

Determination of ion nitrate was carried out using the reduction method. The principle of this method is that cadmium reduces nitrate to nitrite in the sample. Nitrite ions react in acidic conditions with sulfanilic acid and then form diazonium ions. The next step was reading absorption using a spectrophotometer (Shimadzu 3600 UV-VIS) at a wavelength of 540 nm. The analysis of dissolved nutrient P was determined as orthophosphate using the ascorbic acid method by APHA [Maslukah et al., 2019], and the absorbance was determined at a wavelength of 885 nm. The principle of phosphate determination is based on the reaction of phosphate ions with molybdate reagent, ascorbic acid, and potassium antimony tartrate and produces a blue-colored solution.

Furthermore, chlorophyll-a analysis was determined by filtering one liter of a water sample using cellulose filter paper (pore-size $0.45 \mu\text{m}$) and extracted using 10 ml acetone (90%), placed in a refrigerator in the dark for 16 hours, and then centrifuged [Maslukah et al., 2018]. The supernatant was read for absorbance at $\lambda 664$, $\lambda 647$, and $\lambda 630$. A wavelength of 750 was used to control turbidity.

The Chl-a was calculated using the formula by Jeffrey and Humphrey (1975) (Formula 1 and 2):

$$c = 11.85 (\lambda 664) - 1.54 (\lambda 647) - 0.008 (\lambda 630) \quad (1)$$

$$\text{Chl-a (mg/m}^3) = c \times v / V \quad (2)$$

where: $\lambda 664$, $\lambda 647$, $\lambda 630$ – the absorbance measurements at those wavelengths; v – volume of extract (liter); V – sample volume (m^3).

The TRIX index was calculated using the formula applied by Peng [2015] following Formula 3:

$$\text{TRIX} = \text{Log}_{10}(\text{Chl-a} \times [\% \text{DO}] \times \text{DIN} \times \text{SRP} + 1.5) / 1.2 \quad (3)$$

This approach depends on four variables: dissolved oxygen, dissolved inorganic nitrogen (DIN), chlorophyll-a, and dissolved inorganic nitrogen. The percentage of dissolved oxygen (%DO), was calculated as $[100 - \% \text{DIN}]$.

The Kruskal-Wallis test was used to evaluate the data in order to compare the mean nutrient and chlorophyll-a concentrations from various sites. P0.05 was regarded as the significance cutoff. Furthermore, to determine differences between locations, the Bonferroni test was used. The results of the DIP nutrient relationship model with chlorophyll-a using multiple linear regression models. The data were examined using a statistical program with SPSS v.21. The bivariate correlation test was performed to examine the relationship between chlorophyll-a and nutrients (nitrate, ammonium, and phosphate).

RESULTS AND DISCUSSION

Spatial variations of nutrients

Nutrients are critical to aquatic productivity and ecosystem health. The results of research in three locations, namely Jobokuto Bay (Jepara), in the mouth of the BKT dan BKB (Semarang), and in the mouth of the Ciasadane River (Banten) had varying concentrations of N and P nutrients. Each of these areas has a characteristic N/P ratio (stoichiometry ratio). The ion of nitrate ranged from 0.039–0.117 mg N-NO₃/L in Jepara waters with an average value of 0.078 N-NO₃/L, in Semarang coastal waters between 0.010–0.062 N-NO₃/L with an average value of 0.030 N-NO₃/L and in the mouth of Ciasadane river ranged from 0.020-0.035 N-NO₃/L with an average of 0.027 N-NO₃/L. The ion of nitrate in Jepara waters was twice as high as those in Semarang

waters and the Ciasadane estuary. This distribution is different for ammonium. Semarang waters had higher values than Jepara waters. The distribution was similar for phosphate concentrations, which showed higher values in Semarang waters. Phosphate concentrations in Semarang ranged from 0.028–0.036 mg P-PO₄/L with an average of 0.030 mg P-PO₄/L. The details of the data nutrients are presented in Table 1. Based on Table 1, the nutrient fluctuation pattern Chl-a and its stoichiometric ratio from three areas (Jepara, Semarang, and Ciasadane) are presented in Figure 2. Normality tests in nutrient data (N, P) (Table 1) resulted in normal distributions, but not in Chl-a. Consequently, to examine differences in nutrients and Chl-a between locations, we used the Kruskal Wallis test (Table 2) and Spearman correlation between Chl-a and nutrients and their ratios (Table 3). The Kruskal-Wallis test indicates that there are differences in the average nutrient

Table 1. The concentration of NO₃ (mg N-NO₃/L), N-NH₄ (mg N-NH₄/L), P (mg P-PO₄/L), Chl-a (µg/L), and N/P ratio in Jepara, Semarang, and Ciasadane coastal waters

Area	Station	NO ₃ ⁻	NH ₄	DIN	Phosphate	Chl-a	Ratio N/P
Jepara	1	0.117	0.610	0.727	0.010	1.451	2.154
	2	0.116	0.283	0.399	0.019	1.386	0.668
	3	0.039	0.077	0.116	0.016	0.907	0.223
	4	0.049	0.180	0.230	0.007	12.110	0.961
	5	0.067	0.179	0.247	0.017	9.192	0.462
Average		0.078	0.266	0.344	0.014	5.009	0.893
Semarang	1	0.010	0.242	0.252	0.036	41.968	0.447
	2	0.049	0.255	0.304	0.033	19.734	0.682
	3	0.005	0.071	0.076	0.030	23.536	0.727
	4	0.033	0.087	0.120	0.028	14.393	2.791
	5	0.036	0.198	0.235	0.029	11.640	3.494
	6	0.022	0.486	0.508	0.027	17.400	2.564
	7	0.021	0.457	0.478	0.029	14.056	7.361
	8	0.062	1.061	1.123	0.029	11.270	10.290
	9	0.037	0.900	0.938	0.028	7.070	36.642
	10	0.028	2.459	2.487	0.031	29.371	7.663
Average		0.030	0.622	1.196	0.030	19.044	7.266
Ciasadane	1	0.035	-	0.035	0.047	8.983	0.748
	2	0.030	-	0.030	0.033	8.304	0.913
	3	0.027	-	0.027	0.030	8.611	0.901
	4	0.029	-	0.029	0.024	7.806	1.206
	5	0.032	-	0.032	0.020	18.685	1.601
	6	0.020	-	0.020	0.018	8.130	1.148
	7	0.024	-	0.024	0.023	14.596	1.018
	8	0.021	-	0.021	0.019	14.880	1.113
Average		0.027		0.027	0.021	12.819	1.217

Table 2. Post-Hocdoc test of nitrate, DIN, phosphate, and Chl-a in different the mouth of the river

Variable		(I) location	(J) location	Mean difference (I-J)	Std. error
Nitrate	Bonferroni	Jepara	Semarang	-0.5208	0.2785
			Cisadane	-0.805*	0.2899
		Semarang	Cisadane	-0.2844	0.2412
DIN	Bonferroni	Jepara	Semarang	-0.852	0.4107
			Cisadane	-0.5389	0.4275
		Semarang	Cisadane	0.3131	0.3557
Phosphate	Bonferroni	Jepara	Semarang	-0.016*	0.0035
			Cisadane	-0.013*	0.0037
		Semarang	Cisadane	0.0033	0.0031
Chl-a	Bonferroni	Jepara	Semarang	-14.035*	4.231
			Cisadane	-6.2402	4.4034
		Semarang	Cisadane	7.7944	3.6638
Ratio N/P	Bonferroni	Jepara	Semarang	-6.3725	4.8211
			Cisadane	-33.975*	5.018
		Semarang	Cisadane	-27.603*	4.1752

Note: *The mean difference is significant at the 0.05 level).

Table 3. The correlation coefficients of Chl-a with N, P, and N/P ratio

		Location	NO ₃	NH ₄	P	Rasio
Jepara	Chl-a	The coefisien of correlation	.100	.200	-.500	.500
		Sig. (2-tailed)	.873	.747	.391	.391
		n	5	5	5	5
Semarang	Chl-a	The coefisien of correlation	-.624	-.224	.671*	-.721*
		Sig. (2-tailed)	.054	.533	.034	.019
		n	10	10	10	10
Cisadane	Chl-a	The coefisien of correlation	.167	.	-.190	.048
		Sig. (2-tailed)	.693	.	.651	.911
		n	8	0	8	8

nitrate, phosphate, N/P ratio, and Chl-a at each location. The results of the differences at each location are shown in Table 2. In Table 3, the element of P and the N/P ratio of the three regions show significant differences ($p < 0.05$). Those affected the level of chlorophyll-a, which showed a high level in Semarang waters. Furthermore, the bivariate relationship between chlorophyll-a and N, P, and N/P ratio is shown in Table 3.

DISCUSSION

Eutrophication of coastal waters is considered one of the major threats to marine ecosystems. Indicators of trophic conditions can be using Chl-a, which is the major pigment in phytoplankton. The Chl-a reflected the water quality

in the aquatic ecosystem. The spatial Chl-a fluctuates due to the influence of the human activity level in the surrounding area [Damar et al., 2020; Prayitno and Afdal, 2020]. Semarang is the capital city of Central Java, which has the highest population and anthropogenic activities compared to Jepara and Cisadane. Accordingly, Chl-a concentration in Semarang waters (BKT and BKB) reached 7.07–41.98 ug/. The previous research by Maslukah et al. [2019] observed that BKB and BKT Semarang waters had the highest chlorophyll-a concentration compared to other waters such as Kendal, Pekalongan, and several coastal waters in Jepara (Wisio estuary and the surrounding Panjang Island). There are also temporal variations in each region. The Chl-a of Semarang waters was observed to be high in the rainy season, while

for Jepara waters in the dry season. A previous study by Maslukah et al. [2019] explained that the spatial variation of Chl-a in the northern waters of Central Java was related to the nutrient orthophosphate (PO_4). Prayitno and Afdal [2020] also found that Chl-a was more related to nutrient phosphate in Teluk Jakarta.

In addition, variations in each area are affected by the distance from the river flow, which is closely related to nutrient sources [Damar et al., 2020; Prayitno & Afdal, 2020]. In general, from the three research locations, nutrient-N (nitrate and ammonium) were found at stations near the estuary, which in this study we found at station 1 (in front of the Wiso estuary, Jepara), in front of the BKB estuary (station 10) and BKT (station 2) (Semarang) and in front of the mouth of the Cisadane River, at station 1 (Figure 1). A similar pattern was found for phosphate nutrients, except for Jepara waters which were found at station 6 which is located near land. Through the Theoretical Dilution Line (TDL) approach between nutrients and salinity, rivers make the main contribution as a source of nutrients in coastal waters [Maslukah et al., 2014]. Based on Table 1, using the ratio of values from each location, we found that the distribution of Chl-a is closely related to the nutrients including N, P, and the N/P ratio (stoichiometry ratio). The highest Chl-a levels were found in Semarang, followed by Cisadane and Jepara with concentrations of 19.044, 12.819, and 5.009 $\mu\text{g}/\text{m}^3$, respectively. The value of Chl-a in Semarang waters is related to the observed nutrient P, i.e. reaches 0.030 mg/L and the ion of ammonium is 0.622 ppm (Figure 2). In addition, the ratio N

to P (N/P) also shows the highest. However, it is still below his Redfield ratio of 16. The ratio (<16) explains that phosphate ions are found to be more abundant and in this condition, the nutrient N is a limiting factor of biological processes. The results of Moon et al. [2021] explain that N has the same likelihood as P in affecting the growth of phytoplankton. The results of a simulation in the laboratory by Ganguly et al. [2013] find that ammonium and phosphate have provided a positive response to the growth of some microalgae. Nasir et al. [2015] explained that the Makasar coastal waters showed the presence of eutrophication processes associated with $\text{NH}_3\text{-N}$ concentrations in waters reaching 6.37-13.6 μM ($\sim 0.09\text{--}0.19 \text{ mg N/L}$) and a relatively high N/P nutrient ratio in coastal water.

However, based on the correlation test between chlorophyll-a to nutrients (Table 3) at each location, we found only Jepara waters that had a positive relationship to the N/P ratio. This explains that the N/P ratio plays a role in the increase in chlorophyll-a, which is an indicator of the growth of phytoplankton. It differs from Semarang coastal waters that show a positive correlation to the P ion, but Cisadane coastal water does not show a relationship. We estimate that there is a correlation between the process of P ion release from sediments to water columns for the Semarang location based on previous research by Maslukah et al. [2019].

The differences in nutrient N, P, and ratio of N/P between locations are presented in Table 2. Semarang waters, which have high anthropogenic activity, showed higher nutrient P than the other two coastal waters and were followed

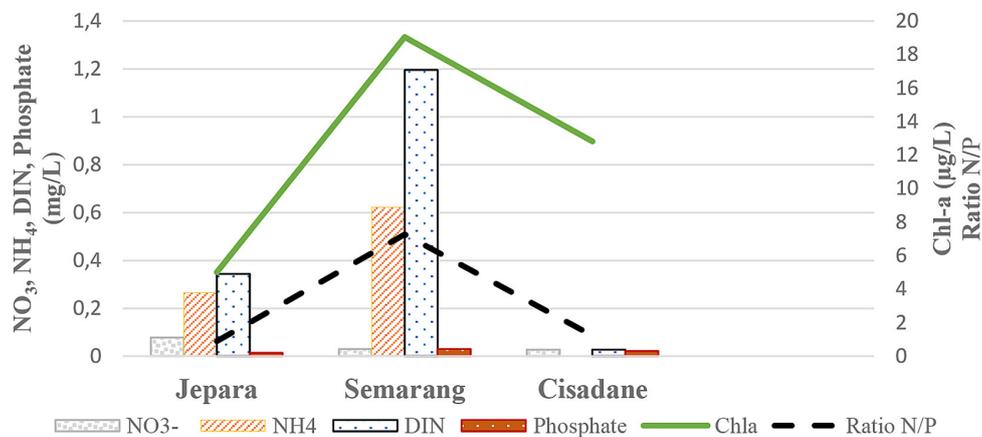


Figure 2. The average values of Nitrate (NO₃), Ammonium (NH₄), Dissolved Nitrogen (DIN), Phosphate (PO₄), Chlorophyll-a (Chl-a) and Stioikiometric Ratio (N/P) in Jepara, Semarang and Cisadane

by increasing N concentration (seen through the N/P ratio). In this study, the speciation of ammonium was higher in Semarang waters compared to nitrate. Ammonium is the result of early degradation of organic matter and was found to be high in an area polluted by organic matter. Prayitno and Afdal [2020] explained that areas that are industrial and dense residential areas as contributors to the elements of N (ammonia) and Phosphate.

The next step is to calculate the trophic index based on the parameters N, P, Chl-a, and oxygen concentration. Water quality indices are an important tool for summarizing complex water quality data. The ecological risk of N and P can be assessed using the index of trophic formula (TRIX). In this study, TRIX was determined according to Vollenweider [Saravi et al., 2019]. Multimetric trophic index (TRIX) can be used to evaluate coastal eutrophication [Al-Mur, 2020]. According to Primpas and Karydis [2011], it takes with consideration the primary effects of eutrophication such as environmental disturbance, biological reaction, and stress response. TRIX was thus employed for this study's assessment reference for coastal eutrophication. The variables used in the calculation of TRIX are those related to primary production (Chl-a and DO), DIN (NO_2^- , NO_3^- , NH_4^+), and P- PO_4 [Saravi et al., 2019; Sá et al., 2021]. Saravi et al., [2019] classified into five category scales for the state of water quality: very good (oligotrophic with $\text{TRIX} < 4$), good (medium trophic level with $4 < \text{TRIX} < 5$), moderate (high trophic with values in the range $5 < \text{TRIX} < 6$), and poor (very high trophic level with $\text{TRIX} > 6$).

The model of TRIX is used to express the state of enrichment and water quality conditions for various elemental components, especially nitrogen, and phosphorus. The TRIXS model has been applied to many waters in both freshwater and coastal seas in several regions in Europe. The results of determining the trophic index in the study area had mean values (Jepara, Semarang, Cisadane) of 4.68, 5.15, and 4.53 respectively. The Jepara and Banten locations are still in good condition, but the coastal waters of Semarang are in the moderate category. Even so, the TRIX value from this study is still lower than the waters of Teluk Jakarta. The coastal waters of Jakarta Bay are dominated by mesotrophic and hypertrophic conditions (TRIX value > 5) [Damar et al., 2020].

CONCLUSIONS

The variation of chlorophyll-a is related to nutrient concentrations, which are influenced by the level of anthropogenic activity. The areas with high anthropogenic activity are the most important sources of nutrient of P, which we found in Semarang waters in this study. The high availability of P has a significant impact on the high Chl-a in these waters. Besides P (phosphate), N also affects the Chl-a. In this study, we showed that a high of ratio N/P (stoichiometric ratio) plays a role in enhancing phytoplankton growth, which in this study we looked at based on biomass indicators, through Chl-a analysis. The dissolved N speciation in the water was in the form of ammonium, which had a greater influence on Chl-a high in Semarang compared to Jepara waters. It is important to measure nutrient N and its speciation in addition to P to monitor eutrophication.

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