

## Impact of Nutrients and Suspended Particulate Matter on Phytoplankton Chlorophyll-a Biomass, in the Estuary of Kendal, Indonesia

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### ABSTRACT

The Kendal River estuary receives nutrient input from various community activities from the surrounding waters and will indirectly affect the physical and biological quality parameters of the waters. Phytoplankton biomass is a parameter of biological quality, which can be measured based on the concentration of chlorophyll-a, while suspended particulate matter (SPM) is a parameter of its physical quality. The purpose of this study was to determine the spatial distribution of nutrients and suspended particulate matter and their effect on chlorophyll-a in the Kendal River Estuary. This research was conducted in November 2017. Sampling was carried out at 12 stations representing river, coastal and high seas areas. Chlorophyll-a and nutrients N & P, were measured based on the spectrometric method and SPM using the gravimetric method. Spatial distribution of each parameter using kriging interpolation with ArcGIS. The results showed that chlorophyll-a, nitrate, phosphate, and SPM had a similar spatial distribution pattern, which was high in the estuary area and decreased in concentration towards the sea. The results of the multiple linear regression model analysis between chlorophyll-a on nutrients and SPM followed the formula  $[\text{chl-a}] = -11275 + 5.249 [\text{NO}_3^-] + 6.987 [\text{PO}_4^{2-}] + 0.004 [\text{SPM}]$ , with a coefficient of determination ( $R^2$ ) of 0.76. The most influential water quality is phosphate, followed by nitrate and SPM. Based on the chlorophyll-a value, Muara Kendal is categorized as eutrophic.

**Keywords:** chlorophyll-a, water quality, Kendal estuary.

### INTRODUCTION

The estuary is a very dynamic environment that is vulnerable to human activities. Thus, monitoring phytoplankton abundance and composition is an essential tool for the prediction of eutrophication and its effects on coastal ecosystems. Human activities have had a significant effect on increasing the input of pollutants into the waters (William et al., 2010; Utami et al., 2015). This area will accommodate waste generated from industrial activities from around the watershed (DAS) and domestic waste from residential areas (Baktiar et al., 2016). This waste

contains organic compounds. The ecological impact of the entry of organic waste from land to river estuaries has made the waters more fertile due to the increase in N and P nutrients (Maslukah et al., 2018).

These nutrients play a role in the processes and development of living organisms such as phytoplankton (Wang et al., 2015; Wisna and Maslukah, 2017). Chlorophyll-a (chl-a) can be used as a measure of phytoplankton biomass (Batali et al, 2013; Damar et al, 2020; Diana et al. 2021). Phytoplankton biomass, as chlorophyll-a in an estuary, varies in response to nutrients and suspended solids (Bucci et al., 2012; Maslukah et

al., 2020). Magumba et al. (2013) explained that nitrogen greatly affects the chlorophyll concentration, especially chlorophyll-a. Chlorophyll-a is a pigment found in all types of phytoplankton (Damar et al., 2020).

The relationship between chlorophyll-a (chl-a) and nutrients in the water column has been studied intensively by several researchers (Søndergaard et al., 2011; Maslukah et al., 2019; Damar et al., 2020). The nutrient concentrations of phosphorus (P) and nitrogen (N), together or in isolation, affect the chl-a concentration (Trommer et al., 2013). The relationship of variation is influenced by factors of latitude, height, depth, and stoichiometric characteristics (Abell et al., 2012). Maslukah et al. (2018) explained that chlorophyll-a in Jepara waters has the strongest correlation with the N/P ratio, followed by P and finally N individually. The high level of human activity on the north coast of Java has affected the input of P nutrients in the waters (Maslukah et al., 2019) and P nutrient can be used in the eutrophication monitoring process. An increase in the P concentration of nutrients from each estuary in the study area, followed by an increase in the concentration of chlorophyll-a. Furthermore, Maslukah et al. (2019) found a relationship between chlorophyll-a and nutrient P following a logarithmic model with the formula  $[\text{chl-a}] = 1.52 + 1.60 \log [P]$ , with a correlation coefficient ( $r$ ) of 0.74. The research on the horizontal distribution of N and P nutrients in the Kendal estuary was carried out by Hanifah et al. (2018). However, its presence has not been linked to chlorophyll-a concentrations. The relationship of water quality (nutrient N, P, and total suspended solids) can be described in the form of a linear relationship model. A comprehensive model of this relationship can be applied to waters with different characteristics and will be invaluable in developing effective policies to control water quality (Abell et al., 2012).

Kendal River is one of the rivers that flows into the northern coastal waters of Java. The Kendal River flows through a relatively densely populated urban area. The area around the mouth of the Kendal River is intensively utilized by the community as residential land, aquaculture, fish auctions, and ports. These various human activities can affect water quality and in turn, can affect the chlorophyll-a concentration in the Kendal estuary waters. Based on these backgrounds, this

study aims to determine responses between chlorophyll-a and nitrate ( $\text{NO}_3^-$ ), phosphate ( $\text{PO}_4^{2-}$ ), and suspended particulate matter (SPM). The results of this study are expected to be used in determining water quality parameters (nitrate, phosphate, and SPM) which significantly affect the abundance of phytoplankton through the measurement indicator chlorophyll-a.

## MATERIALS AND METHODS

This research was conducted at the Kendal estuary in the rainy season (November 2017) at  $110^{\circ}13'44.09''$ - $110^{\circ}14'5.96''$ E Long. and  $6^{\circ}53'16.9''$ - $6^{\circ}52'47.97''$ S Lat. The parameters studied were suspended particulate matter and chlorophyll-a. The nutrient data ( $\text{NO}_3\text{-N}$  &  $\text{PO}_4\text{-P}$ ) used in this analysis were the results of research by Hanifah et al. (2018) at the same location. The station was determined based on a purposive sampling method from 12 stations with the consideration of representing the river, coastal, and open sea areas. Seawater samples were taken as much as 500 ml (for SPM) and 1000 ml (for chlorophyll-a) and stored in a cool box to stop the biological activity.

Determination of the concentration of chlorophyll-a – water samples were filtered using a Millipore filter membrane (pore size  $0.45 \mu\text{m}$ ). The sample was added 3-5 drops of 1%  $\text{MgCO}_3$ , during the sample filtering process. The suspended (plus filter paper) was extracted with 10 ml 90% acetone and stored in the refrigerator for 16 hours. Next, centrifugation is carried out to make the extracted chlorophyll-a (greenish colored liquid) from the solid. This process lasts 30 minutes at a speed of 4000 rpm. The top extraction result was poured into a cuvette and the absorbance value was read at a wavelength of 664 nm, 647 nm, 630 nm, using UV-Vis Optima, with 1 cm wide cuvet. The concentration of chlorophyll-a was calculated using formula (1) (APHA, 1992):

$$Ca = (11.85 \times E_{664}) - (1.54 \times E_{647}) - (0.08 \times E_{630}) \quad (1)$$

where:  $Ca$  – concentration of chlorophyll-a in extract;  $E$  – absorbance at wavelength corrected for wavelength 750 nm.

After determining the chlorophyll-a concentration in the extract, the total chlorophyll-a concentration per unit volume was calculated as follows (Jefrey & Humphrey, 1975), according to formula (2):

$$\text{Chlorophyll-a} = \frac{Ca \times Va}{V \times d} \text{ (mg} \cdot \text{m}^{-3}\text{)} \quad (2)$$

where: *Ca* – concentration of chlorophyll-a in extract (mg/m<sup>3</sup>);  
*Va* – volume of acetone (ml);  
*V* – volume of filtered water sample (L);  
*d* – cuvet diameter (1 cm).

Determination of suspended particulate matter – about 1 liter of seawater was filtered through pre-weighed Whatman filters paper (type 42) for suspended sediment analysis. Filter paper and suspended drying in an oven at ±105 °C for 1 hour. After drying it is put into a desiccator and weighed. SPM calculations using formula 3 (Alaerts & Santika, 1989):

$$\text{SPM} = \frac{a-b}{c} \text{ (mg/l)} \quad (3)$$

where: *a* – weight of filter paper and residue (mg);  
*b* – weight of initial filter paper (mg);  
*c* – volume of water sample (liter).

The analysis of data – a geostatistical tool, kriging, is used in ArcGIS to spatially interpolate measured values for the chlorophyll-a, SPM, and nutrient variables. The multiple linear regression test was conducted to analyze the interaction between NO<sub>3</sub><sup>-</sup>, PO<sub>4</sub><sup>2-</sup> and SPM in influencing chlorophyll-a pigment. Normality tests were performed on all data, before further analysis of the regression model. The model criteria use the coefficient of determination (R<sup>2</sup>) and the correlation coefficient (r) is used to determine the strength of the relationship. The significance test was carried out at p < 0.05.

## RESULTS AND DISCUSSION

### The distribution pattern of chlorophyll-a and total suspended solids

The results showed that the chlorophyll-a and TSS concentrations in the Kendal estuary were between 2.41–17.67 mg/m<sup>3</sup> dan 131–434 mg/l. The horizontal distribution patterns of chlorophyll-a and SPM are shown in Figure 1 and Figure 2.

### The nutrients distribution pattern of nitrate and phosphate

The concentrations of nitrate and phosphate used in the analysis of this study were the results of research by Hanifah et al. (2018) at the same location and station. Based on the results of this research, it was found that the nitrate value ranged from 2.12–3.25 μM and the phosphate concentration ranged from 0.17–1.04 μM. The distribution patterns of nitrate and phosphate are presented in Figure 3.

Figure 2, explains that the highest chlorophyll-a concentration at station 3 (17.67 mg/m<sup>3</sup>). The highest concentration of chlorophyll-a at this station is due to its location at the mouth of the river and close to the mainland. Inland areas generally have high concentrations of nutrients, which originate from human activities on land and accumulate in these areas through river runoff. This is evidenced by the research of Hanifah et al. (2018) in Figure 3, that station 3 has the highest nitrate concentration reaching 3.25 μM. There is another process that can

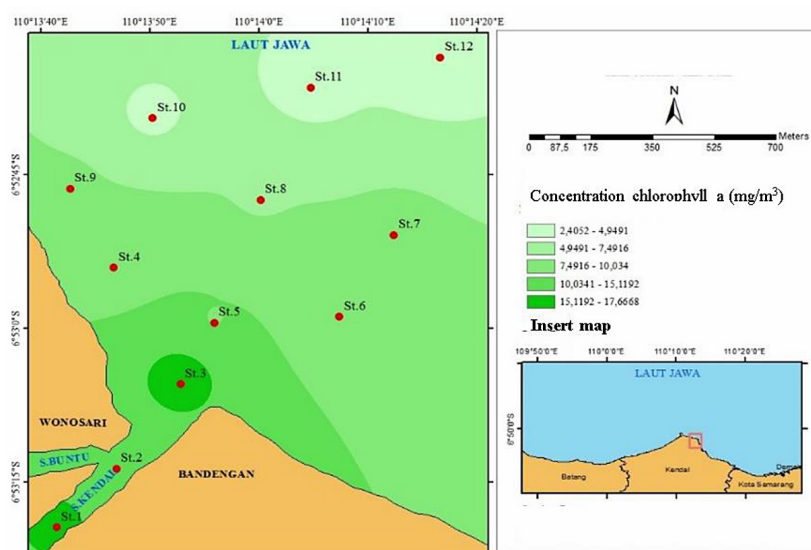


Figure 1. The distribution pattern of chlorophyll-a in Kendal Estuary

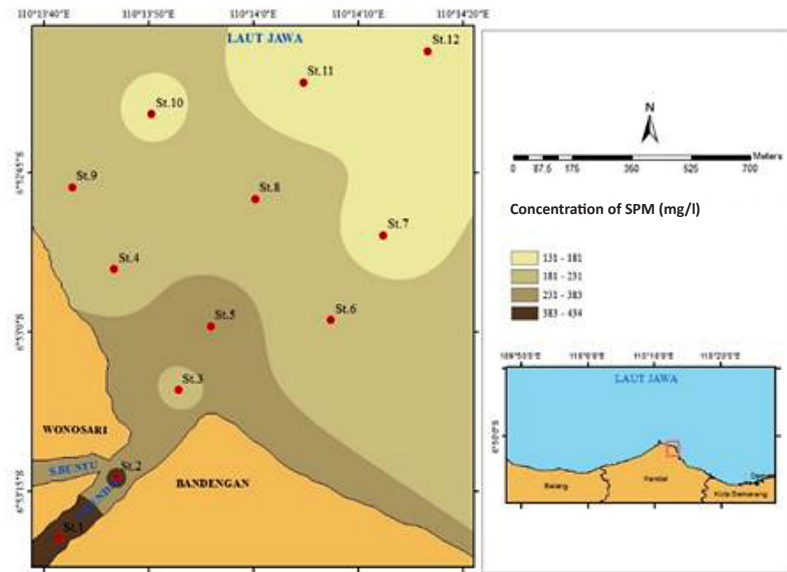


Figure 2. The distribution pattern of suspended particulate matter in Kendal Estuary

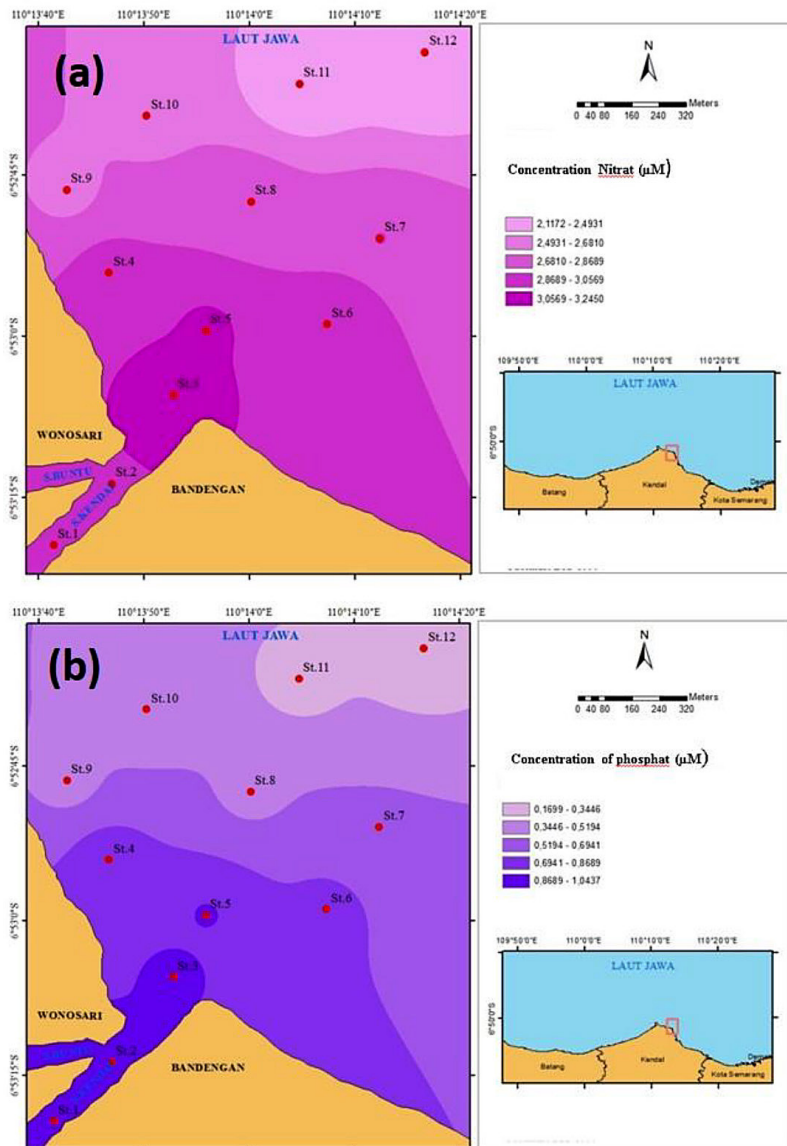


Figure 3. The distribution pattern of (a) nitrate and (b) phosphate in estuary Kendal (Hanifah et al., 2018)



increase the high concentration at the station, namely sediment resuspension. Sediment resuspension is related to the current velocity at the station. The Kendal River is used by the community for various activities, including a residential area, aquaculture, a fish auction, and a port where fishing boats leaned. These human activities produce waste containing organic compounds. The result of the degradation process of organic matter, nutrients including  $\text{NO}_3$  and  $\text{PO}_4$ . Rahmawati et al. (2014) explained that the high concentration of chlorophyll-a in the estuary was caused by the higher nutrient input from the land carried by the river flow to the river estuary. Besides, estuaries are a relatively frequent area of churning due to the confluence of fresh water and seawater. This stirring process can cause nutrient enrichment from the bottom to the surface of the water. As evidence that station 3 has a resuspension process, it can be seen from the high SPM concentration at that station, reaching 223 mg/l. SPM is a suspended material floating in the water and consists of biotic components (phytoplankton, bacteria, and fungi) and abiotic components (detritus and inorganic particles) (Satriadi and Widada, 2004).

Figure 2 also shows that the lowest chlorophyll-a concentration is at station 12, which is 2.41 mg/m<sup>3</sup>. Station 12 is located in the open sea and far from the mainland, so the stirring process is minimal. Station 12 has a relatively high brightness value reaching 200 cm and a relatively low SPM concentration (131 mg/l) when compared to other stations. Low chlorophyll-a concentrations are associated with locations far from nutrient sources (land) so that this area receives little nutrient input. Hanifah et al. (2018) explained that the concentrations of nitrate and phosphate at station 12 were 2.12  $\mu\text{M}$  and 0.17  $\mu\text{M}$ , respectively. The low chlorophyll-a in the open sea is due to the lack of direct sources of nutrients from the land. Maslukah et al. (2018) found a very strong positive correlation between the ratio of N/P nutrients to chlorophyll-a. Based on the average of 12 stations, the chlorophyll-a concentration in the Kendal estuary is 8.68 mg/m<sup>3</sup>. Thus, these waters can be categorized as eutrophic. According to Hakanson and Bryann (2008) chlorophyll-a concentrations of 6–20 mg/m<sup>3</sup> are categorized into eutrophic. The eutrophic category means that the waters are fertile with an abundance of nutrients (nitrate and phosphate). The results of

research by Maslukah et al. (2019) state that the research location in the urban center affects the high nutrient and chlorophyll-a values. This is caused by the discharge of waste from population activities in the form of nitrogen compounds that come from metabolic waste.

The results of the analysis of multiple linear regression models between chlorophyll-a to  $\text{NO}_3$ ,  $\text{PO}_4$  and SPM in this study follow the formula:  $[\text{chl-a}] = -11.275 + 5.249 [\text{NO}_3] + 6.987 [\text{PO}_4] + 0.004 [\text{SPM}]$ . The multiple linear relationship model of chlorophyll-a to  $\text{NO}_3$ ,  $\text{PO}_4$ , and SPM can be described by the coefficient of determination ( $R^2$ ) of 0.79. This explains that the presence of chlorophyll is strongly influenced by 79% by the presence of  $\text{NO}_3$ ,  $\text{PO}_4$ , and SPM, at a very significant level of significance ( $p = 0.004$ ). To see the relationship of each variable to the chlorophyll value can be seen from the correlation test (Table 1). Based on the correlation Table 1, it was found that nitrate ( $\text{NO}_3$ ) and phosphate ( $\text{PO}_4$ ) had a significant effect at the level of  $p < 0.01$  and SPM had a significant effect at the level of  $< 0.05$ . This explains that the distribution of chlorophyll-a concentrations in the estuary Kendal is strongly influenced by the high nutrient input from activities on the nearby land. Paudel et al. (2019) explained that there is a strong correlation between phosphate and chlorophyll, but it becomes low in low suspended conditions. In the study by Muslim & Jones (2003) at Nelly Bay, a significant positive correlation was found between chlorophyll a to phosphate and SPM, and conversely, by Fanella (2019) showed a negative correlation between SPM and chlorophyll-a ( $r = -0.6376$ ). The negative correlation indicates that high SPM, low chlorophyll-a, and positive correlation indicates the contribution of organisms (phytoplankton). To see the linear regression equation for chlorophyll-a with each parameter, it can be seen in Table 2. The study by Muslim and Jones (1993) in Nelly bay, Australia found the correlation of chlorophyll-a to SPM was 0.49 while chlorophyll-a to phosphate was 0.47. While research by Maslukah et al (2019), in the Java Sea obtained a correlation coefficient of 0.74 and the model follow formula is  $[\text{chl-a}] = 1.52 + 1.60 \log [\text{P}]$ , with  $R^2 = 0.55$ . The TSS value is related to the turbidity value of water. Therefore, its presence also affects chlorophyll-a fluctuations (Bucci et al., 2012).

**Table 1.** Correlation (r) of the relationship between chlorophyll, nitrate, phosphate, and SPM

Component	Correlation	Nitrate	Phosphate	Chl-a	SPM
Nitrate	Pearson Correlation	1	.923**	.860**	.606*
	Sig. (2-tailed)		.000	.000	.037
	N	12	12	12	12
Phosphate	Pearson Correlation	.923**	1	.880**	.812**
	Sig. (2-tailed)	.000		.000	.001
	N	12	12	12	12
Chl-a	Pearson Correlation	.860**	.880**	1	.683*
	Sig. (2-tailed)	.000	.000		.014
	N	12	12	12	12
SPM	Pearson Correlation	.606*	.812**	.683*	1
	Sig. (2-tailed)	.037	.001	.014	
	N	12	12	12	12

**Note:** \*\* correlation is significant at the 0.01 level (2-tailed), \* correlation is significant at the 0.05 level (2-tailed).

**Table 2.** Simple linear regression equation of chlorophyll a to nitrate, phosphate, and SPM

Linear Regression Equation	Coefficient determination (R <sup>2</sup> )
Chl a = -22.693 + 11.234 [NO <sub>3</sub> ], p = 0.000	R <sup>2</sup> = 0.774
Chl a = -0.022 + 13.828 [PO <sub>4</sub> ], p = 0.000	R <sup>2</sup> = 0.740
Chl a = 0.883 + 0.034 [SPM], p = 0.014	R <sup>2</sup> = 0.467

## CONCLUSION

Based on the results of the study, the concentration of chlorophyll-a in the Kendal estuary ranged from 2.41 to 17.67 mg/m<sup>3</sup>. The horizontal distribution of chlorophyll-a was found to be higher in the area near the land (estuary) and gradually reduced towards the open sea. The average concentration of chlorophyll-a from 12 stations was 8.68 mg/m<sup>3</sup> and the Kendal estuary was classified as eutrophic. The multiple linear regression model between chlorophyll-a on water quality (NO<sub>3</sub><sup>-</sup>, PO<sub>4</sub><sup>2-</sup> and SPM) follows the formula [chl-a] = -11.275 + 5.249 [NO<sub>3</sub>] + 6.987 [PO<sub>4</sub>] + 0.004 [SPM], with a coefficient of determination and significance R<sup>2</sup> = 0.790, p = 0.004, respectively.

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