

## Effects of Aquatic Physicochemical Parameters Variation on the Phytoplankton Abundance and Diversity in the Babon River, (Semarang, Central Java, Indonesia)

HAERUDDIN HAERUDDIN\*, FRIDA PURWANTI,  
ARIF RAHMAN and KUKUH PRAKOSO

Department of Aquatic Resources, Faculty of Fisheries and Marine Sciences, Diponegoro University,  
Semarang, Central Java, Indonesia.

### Abstract

The Babon River is an integral component of Semarang City's drainage system, which is heavily impacted by the influx of waste, leading to elevated water temperatures caused by global warming and decreased pH levels due to acid rain. The two main environmental factors that affect phytoplankton growth and productivity are temperature and nutrient availability. Nutrients can change the balance of the natural food webs of aquatic ecosystems. Meanwhile, metals are toxic and can accumulate in biota tissues. This study examined how nutrients, pH, temperature, and heavy metals in water affected the diversity and abundance of phytoplankton. Water samples and phytoplankton were collected from 7 observation stations representing the upstream, middle, and downstream rivers. Observations at each station were made 3 times in the months of April-May, June-July, and August-September. Temperature and pH were measured in situ with the HORIBA Water Checker. Analysis of nitrate, phosphate, and heavy metals was carried out with an AAS. The results showed that the dominant phytoplankton in the Babon Rivers consisted of Bacillariophyceae, Chlorophyceae, and Cyanophyceae. There is no diversity against all independent variables (temperature, pH, nitrate, phosphate, Cd, Total Cr, Pb concentrations) and dependent variables (number of genera, abundance, and diversity index of phytoplankton) due to the sampling station's location. However, sampling time caused diversity in nitrate, phosphate, Cd, Total Cr, and Pb concentrations, the sampling location effect was more dominant than sampling time, so the results showed that all independent variables did not contribute significantly to the dependent variables.



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**CONTACT** Haeruddin Haeruddin ✉ haeruddindaengmile@lecturer.undip.ac.id 📍 Department of Aquatic Resources, Faculty of Fisheries and Marine Sciences, Diponegoro University, Semarang, Central Java, Indonesia.



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## Introduction

Rivers are essential freshwater ecosystems that support a variety of living organisms, provide habitats for aquatic life, promote tourism, facilitate aquaculture, enable water transportation, serve as waste disposal sites, and act as sources of drinking water.<sup>1,2</sup> Increasing water intensively could degrade water quality,<sup>3,4</sup> due to contamination of various materials, such as nutrients,<sup>5,6</sup> and heavy metals.<sup>7-16</sup>

Nutrients, especially N and P, can cause a decrease in water quality, eutrophication, and changes in the balance of the natural food webs of aquatic ecosystems.<sup>17-19</sup> Meanwhile, metals are toxic, difficult to decompose, and can accumulate in biota tissues.<sup>20,21</sup> River water pollution in various parts of the world by nutrients and metals has become a serious concern for multiple groups.<sup>22-29</sup>

Nutrients in water come from various sources, such as agricultural, industrial, and household activities.<sup>22-24,30</sup> Heavy metals in waters come from rock leaching, farming, mining, and industrial activities.<sup>31-33</sup> Heavy metals enter the body system of aquatic biota from various pathways<sup>34-36</sup>

The Babon Watershed is one of the watersheds in Central Java that plays a crucial role in the system's sustainability in the Eastern Semarang region, traversing through the Semarang Regency, Semarang City, and Demak Regency.<sup>37</sup> The flow of the Babon River originates from several tributaries from Mount Butak in Ungaran, Semarang Regency. The Babon watershed consists of three sub-watersheds, namely the Gung sub-watershed (8,371.97 Ha), the Pengkol sub-watershed (7,009.65 Ha), and the Babon downstream sub-watershed (9,201.76 Ha) with the main river length of 33.76 km.<sup>38</sup> Due to its function as a drainage channel for Semarang City, the Babon River receives a lot of waste input from surrounding activities such as agriculture, industry, and households.<sup>39</sup>

The composition and population dynamics of plankton can serve as a reliable instrument for biomonitoring studies to evaluate the water quality of aquatic bodies.<sup>40</sup> A minor change in physico-chemical parameters can influence primary production.<sup>41</sup> The temperature and nutrient supply

are key environmental factors that influence phytoplankton growth and productivity.<sup>42-44</sup>

The concentration of CO<sub>2</sub> and other gases in the air from transportation, industrial, household, and agricultural activities, causes an increase in air temperature and the occurrence of acid rain, which can increase the temperature and lower the pH of river water. Different changes in phyto and zooplankton potentially resulted from both the effect of acidification due to pH decline and warming due to rising temperatures promoting the growth of phytoplankton over zooplankton.<sup>45</sup> This study analyzed the effect of temperature, pH, nutrients, and metals in waters on the composition, dynamics, and structure of phytoplankton communities in the Babon River. So far there is still rarely research conducted to examine the effects of the interaction of temperature, pH, nutrients, and various heavy metals dissolved in water, on the composition, dynamics, and structure of phytoplankton communities in rivers.

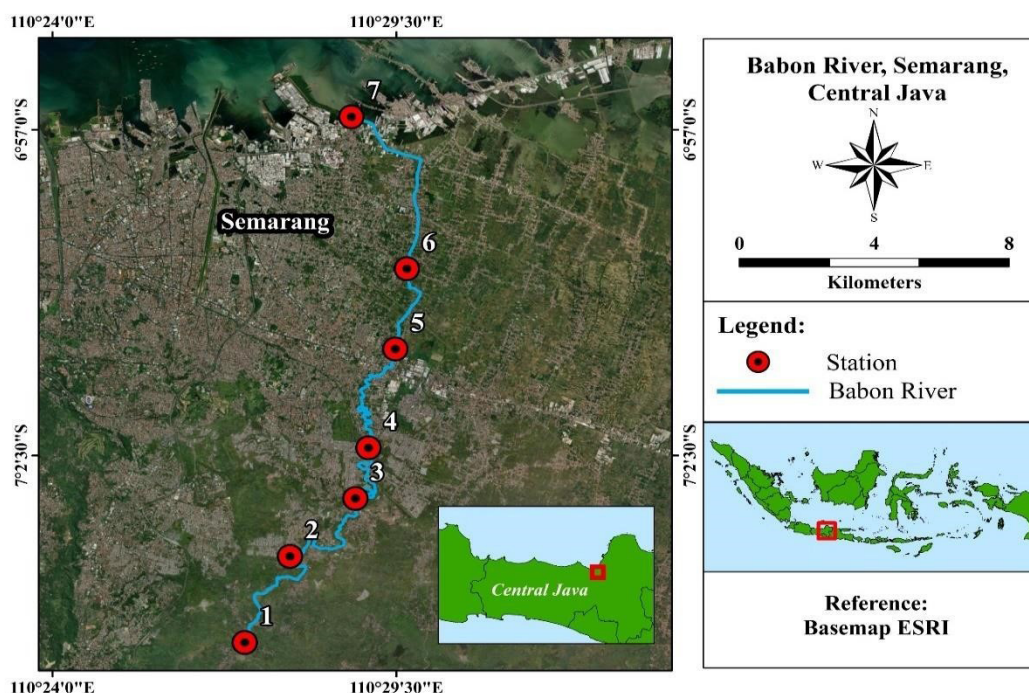
## Materials and Methods

Research materials include water samples and phytoplankton in the Babon River water. The Horiba Water Checker measures the temperature and pH of river waters in situ. Water samples were collected to analyze nutrient (nitrate and phosphate) and metal (Cd, total Cr, and Pb) concentrations in river water. Phytoplankton samples were collected for phytoplankton composition, abundance, and diversity analysis. Water samples and phytoplankton were collected from 7 observation stations (Figure 1.) representing the upstream (stations 1 and 2), middle (3, 4, and 5), and downstream of the river (6 and 7). The sample collection was carried out during April-May (AM), June-July (JJ), and August-September (AS) 2021. The sample collection was carried out in the morning – afternoon, 1 day each for stations 1–4 and 5–7. Water samples at each station were collected from 3 points (left, center, and right), and then combined into 1 sample for each station.

Water samples were collected using Kemmerer bottle for further storage in sample bottles. All sample bottles were placed in a cooler box during transport to the laboratory to analyze NO<sub>3</sub>-N, phosphate, Cd, Cr, and Pb using the APHA method.<sup>46</sup>

The collection of phytoplankton was accomplished by filtering 100 liters of canal water with a plankton trap mesh size of 35  $\mu\text{m}$ . The filtered phytoplankton

was transferred into a sample bottle and fed with a preservative Lugol solution.



**Fig. 1: Sampling stations on the Babon River**

The analysis of nitrate and phosphate was carried out with a Shimadzu Atomic Absorption Spectrophotometer (AAS)-6200 according to APHA (part 4500NO<sub>3</sub>) for nitrate,<sup>46</sup> and APHA (part 4500-P).<sup>46</sup> Analysis of Cd, Total Cr, and Pb was carried out with AAS (APHA, part 3110).<sup>46</sup> Phytoplankton were identified through the use of microscopes and reference books. The abundance of phytoplankton was determined using the Sedwick Rafter Count (SRC), while phytoplankton diversity was established using the diversity index.

The collected data is analyzed statistically utilizing suitable software. The data were divided into independent variables (temperature, pH, nitrate, phosphate, Cd, Total Cr, and Pb concentrations) and dependent variables (number of genera, abundance, and phytoplankton diversity index). All variables were analyzed to determine the presence of variations due to the influence of station location, sampling time, and interaction between station

location and sampling time. In case the data satisfies the statistical prerequisites for the parametric test, ANOVA is conducted with station location as a factor and sampling time as a block. If the statistical criteria are not met, nonparametric tests are utilized instead. Furthermore, a regression analysis was conducted to fix the contribution of each independent variable to each dependent variable. Previously Principal Component Analysis (PCA) was carried out to simplify a large data set into a smaller set.

## Results and Discussion

### Results

The data obtained from the measurement of the temperature and pH of river water are presented in Tables 1 and 2. The concentrations of nutrients (nitrate and phosphate) and metals (Cd, Cr, and Pb) are as follows: the lowest and highest nitrate concentrations are 0.125 mg/l<sup>-1</sup> and 0.57 mg/l<sup>-1</sup>, respectively. The lowest phosphate concentration is 0.008 mg/l<sup>-1</sup>, and the highest is 0.383 mg/l<sup>-1</sup> (Table 4),

While the lowest Cd concentration ( $\text{mg l}^{-1}$ ) was 0.0005 and the highest was 0.14 (Table 5), the lowest total Cr concentration ( $\text{mg l}^{-1}$ ) was 0.0005 and the highest was 0.648 (Table 6). The lowest concentration of Lead ( $\text{mg l}^{-1}$ ) was 0.125, and the highest was 0.57 (Table 7).

**Table 1: Average water temperature of the Babon River during the study**

Sampling time	Sampling stations						
	1	2	3	4	5	6	7
AM	27,37	29,43	29,7	28,13	31,17	30,1	28,53
JJ	26,1	27,13	28,17	28,67	25	29,3	29
AS	23,87	25,07	26,87	31,4	28,97	28,5	27

**Table 2: Average pH of the Babon River During the Study**

Sampling time	Sampling stations						
	1	2	3	4	5	6	7
AM	8.09	7.95	7.36	7.9	7.66	7.62	7.41
JJ	8.29	8.07	7.89	7.99	7.45	7.42	7.31
AS	8.06	8.01	7.66	8.3	7.78	7.81	7.72

**Table 3: Concentration of nitrate ( $\text{mg l}^{-1}$ ) of the Babon River During the Study**

Sampling time	Sampling stations						
	1	2	3	4	5	6	7
AAM	4.120	5.010	4.850	5.230	5.230	5.240	5.410
JJ	0.616	0.734	1.172	0.856	0.968	0.670	0.616
AS	12.679	1.108	2.106	1.578	0.851	0.530	0.381

**Table 4: Phosphate concentration ( $\text{mg l}^{-1}$ ) of the Babon River During the Study**

Sampling time	Sampling stations						
	1	2	3	4	5	6	7
AM	0.346	0.346	0.354	0.383	0.317	0.319	0.277
JJ	0.025	0.026	0.027	0.026	0.029	0.026	0.026
AS	0.165	0.008	0.070	0.080	0.054	0.008	0.008

**Table 5: Cadmium concentration (mg l<sup>-1</sup>) of the Babon River During the Study**

Sampling time	Sampling stations						
	1	2	3	4	5	6	7
AM	0.0005	0.006	0.047	0.068	0.089	0.12	0.14
JJ	0.0005	0.009	0.01	0.008	0.021	0.033	0.042
AS	0.0005	0.0005	0.0005	0.0005	0.0005	0.0005	0.0005

**Table 6: Total Cr concentration (mg l<sup>-1</sup>) of the Babon River During the Study**

Sampling time	Sampling stations						
	1	2	3	4	5	6	7
AM	0.609	0.287	0.185	0.189	0.293	0.342	0.544
JJ	0.0005	0.0005	0.171	0.648	0.394	0.434	0.457
AS	0.0005	0.0005	0.0005	0.0005	0.0005	0.0005	0.0005

**Table 7: Concentration of Lead (mg l<sup>-1</sup>) of the Babon River During the study**

Sampling time	Sampling stations						
	1	2	3	4	5	6	7
AM	0.160	0.152	0.203	0.139	0.223	0.163	0.125
JJ	0.392	0.334	0.316	0.329	0.464	0.325	0.305
AS	0.134	0.243	0.295	0.371	0.57	0.482	0.503

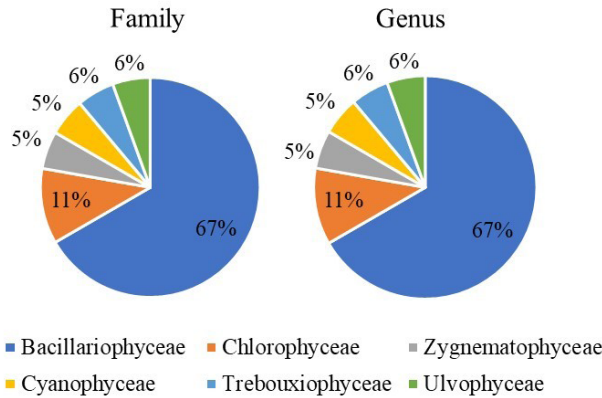
The normality test conducted on the data temperature, pH, nitrate, phosphate, Cd Total Cr, and Pb concentrations using the Ryan-Joiner/Kolmogorov-Smirnov method showed that all the data were not distributed normally, so the analysis of variance was carried out using Friedman's method, where the sampling station as a treatment and blocked by sampling time. The study results showed that the temperature, nitrate, phosphate, Cd, Total Cr, and Pb concentrations between the stations weren't real differences between sampling stations ( $p > 0.05$ ). And that only pH was a real difference between stations ( $p < 0.05$ ). However, if the analysis of variance was carried out using Friedman's method, where the sampling time was a treatment and blocked by the sampling station, different results of the study temperature and pH not a significant difference between sampling time, there were notable variances in nitrate, phosphate, Cd, Cr, and Pb levels across different sampling times.

The dominant class of phytoplankton is the class Bacillariophyceae. The number of families and genera detected in sampling I was 12, sampling II was 15 and 18 respectively, and sampling III was 11 and 18. The types of phytoplankton found during the study are presented in Figures 2, 3, and 4.

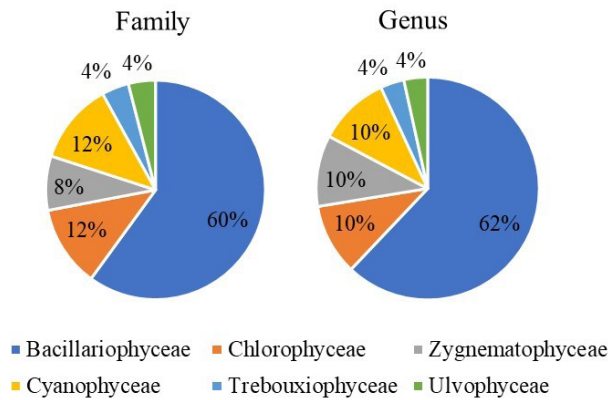
The total count of phytoplankton genera identified across all stations during 3 sampling periods amounted to 30 genera. However, not all genera were discovered at the same time and location during sampling, with some stations only containing 3 genera (lowest) to 16 genera (highest). The lowest number of genera was found at station 4 in sampling 1, while the highest number was at stations 1, 2, 6, and 4 in sampling 2 (Table 8). The lowest phytoplankton abundance is 40 cells/l and the highest is 2545 cells/l (Table 9). The most abundant type of phytoplankton is the genus *Navicula*, followed

by *Pediastrum* and *Nitzschia*. The rarest types found are the genera *Coscinodiscus*, *Eunotia*, and

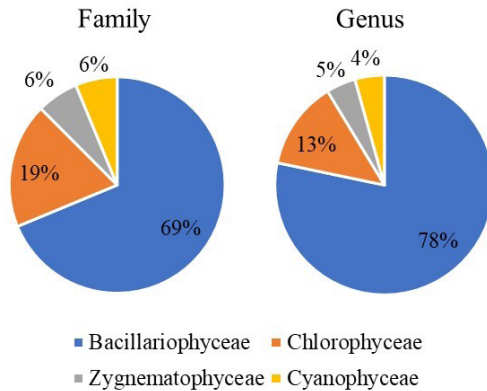
*Rhopadolia*. The lowest diversity index was 0.52 and the highest was 2.12.



**Fig. 2:** The sum of families and genera of phytoplankton are found in sampling I.



**Fig. 3:** The sum of families and genera of phytoplankton are found in sampling II.



**Fig. 4:** The sum of families and genera of phytoplankton are found in sampling III.

**Table 8: Number of genera of phytoplankton on the Babon River During the study**

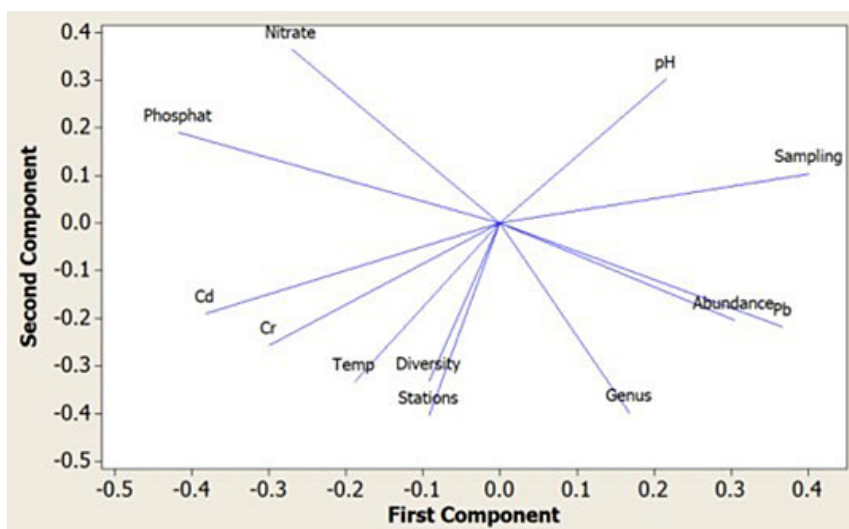
Sampling time	Sampling stations						
	1	2	3	4	5	6	7
AM	9	5	9	3	9	12	6
JJ	16	16	15	13	6	16	13
AS	9	5	6	8	10	13	5

**Table 9: Abundance (ind. l<sup>-1</sup>) of phytoplankton on the Babon River During the study**

Sampling time	Sampling stations						
	1	2	3	4	5	6	7
AM	85	40	160	65	245	420	45
JJ	1805	2545	1395	770	155	675	995
AS	830	145	180	985	570	1060	535

**Table 10: Phytoplankton Diversity Index on the Babon River During the study**

Sampling time	Sampling stations						
	1	2	3	4	5	6	7
AM	2.04	1.39	1.94	0.93	1.8	1.94	1.74
JJ	2.12	1.99	1.95	1.91	1.37	2.12	1.84
AS	1.3	1.17	1.4	1.84	0.52	1.3	1.17



**Fig. 5: Loading plot of the sampling stations, sampling time, and physicochemical parameters**

The results of the Ryan-Joiner/ Kolmogorov-Smirnov Normality Test showed that the number of genera, abundance, and diversity index of all data were not normally distributed, so the variance analysis was carried out using Friedman’s method, in which the sampling station as a treatment and blocked by sampling time. The analysis revealed that there weren’t real differences between sampling stations in several genera, abundance, or diversity index. Nevertheless, when the analysis of variance was conducted utilizing Friedman’s method, with sampling time as the treatment and blocked by the sampling station, it was revealed that there were notable discrepancies in abundance and diversity index across different sampling times, while the number of genera did not exhibit significant differences.

Figure 5 displays a loading plot showcasing the variables that shared the same quadrant: nitrate with phosphate, pH with sampling time, number of genera, phytoplankton abundance with Pb concentration, temperature, Cd, and Cr concentration, and diversity index.

The pattern of relationships between the independent variables (temperature, pH, concentration: nitrate, phosphate, Cd, Cr, and Pb) and the dependent variables (number of genera, abundance, and phytoplankton diversity index) is as follows.

1). The pattern of relationships between the independent variables (temperature (X1), pH (X2), concentration: nitrate (X3), phosphate (X4), Cd (X5), Cr (X6), and Pb (X7) and the dependent variables (number of genera (Y1) are:  $Y1 = - 31.0 + 0.846 X1 + 2.54 X2 + 0.229 X3 - 24.7 X4 + 8.3 X5 + 4.14 X6 - 4.2 X7$

**The values of the coefficients, SE, T, and P of each variable are as follows**

Predictor	Coef	SE Coef	T	P
Constant	-30.97	37.51	-0.83	0.424
Temp	0.8463	0.6267	1.35	0.200
pH	2.540	4.160	0.61	0.552
Nitrate	0.2292	0.5506	0.42	0.684
Phosphate	-24.66	12.44	-1.98	0.069
Cd	8.26	35.92	0.23	0.822
Cr	4.143	5.220	0.79	0.442
Pb	-4.19	13.85	-0.30	0.767

The pattern of relationships between the independent variables (temperature (X1), pH (X2), concentration: nitrate (X3), phosphate (X4), Cd (X5), Cr (X6), and Pb (X7) and the dependent variables (abundance and phytoplankton (Y2) are:

2). The relationship pattern between the independent variables (temperature (X1), pH (X2), concentration: nitrate (X3), phosphate (X4), Cd (X5), Cr (X6) and Pb (X7)) and the dependent variables (abundance and phytoplankton (Y2)) was:  $Y2 = - 7727 + 64.9 X1 + 923 X2 + 6.6 X3 - 3503 X4 + 3846 X5 - 408 X6 - 649 X7$

**The coefficients, SE, T, and P for each variable were as follows:**

Predictor	Coef	SE Coef	T	P
Constant	-7727	5206	-1.48	0.162
Temp	64.93	86.99	0.75	0.469
pH	922.6	577.4	1.60	0.134
Nitrate	6.65	76.43	0.09	0.932
Phosphate	-3503	1727	-2.03	0.064
Cd	3846	4987	0.77	0.454
Cr	-408.2	724.6	-0.56	0.583
Pb	-649	1923	-0.34	0.741

3). The relationship pattern between the independent variables (temperature (X1), pH (X2), concentration: nitrate (X3), phosphate (X4), Cd (X5), Cr (X6) and Pb (X7)) and the dependent variables (diversity index (Y3)) was:  $Y3 = - 1.50 + 0.0763 X1 + 0.237 X2 - 0.0292 X3 - 1.76 X4 + 0.38 X5 + 0.643 X6 - 2.37 X7$

**The coefficients, SE, T, and P values of each variable are:**

Predictor	Coef	SE Coef	T	P
Constant	-1.501	3.696	-0.41	0.691
Temp	0.07626	0.06176	1.23	0.239
pH	0.2369	0.4100	0.58	0.573
Nitrate	-0.02920	0.05427	-0.54	0.600
Phosphate	-1.756	1.226	-1.43	0.176
Cd	0.383	3.541	0.11	0.915
Cr	0.6429	0.5145	1.25	0.233
Pb	-2.368	1.365	-1.73	0.107



## Discussion

Previous studies have demonstrated that water quality parameters, such as Research on the effects of various physic-chemical environmental factors on phytoplankton population dynamics in rivers has been widely conducted.<sup>40,41,47-49</sup> The researchers got similar results to the previous researchers' findings, but some are different. as nutrients and water temperature, are the primary factors influencing the succession processes of phytoplankton communities.<sup>50,51</sup> However, these parameters are susceptible to modification due to watershed land use and climate change.<sup>52</sup> Water quality, specifically NO<sub>3</sub>-N, TAN, TSS, and TUR, has been shown to have significant impacts on phytoplankton abundances, biomasses, species richness, and diversity.<sup>53</sup> The results of this study showed that the independent variables studied (temperature, pH, nitrate concentration, phosphate, Cd, Cr, and Pb) and dependent variables (number of genera, abundance, and phytoplankton diversity index) did not differ markedly between sampling stations. However, there were significant differences between sampling times for the variables nitrate, phosphate, Cd, Cr, and Pb concentrations, number of genera, and phytoplankton abundance. Variable temperature, pH, and diversity index did not differ markedly between sampling times. Thus, it can be stated that the variation in the concentration of nitrate, phosphate, Cd, Cr, and Pb concentrations, number of genera, and phytoplankton abundance is more due to different environmental conditions at different sampling times than at different sampling locations. We particularly believed it was connected to varying precipitation, impacting the flow of the Babon River. In sampling 1 (April – May), rainfall ranged from 131 – 205 mm, with the number of rainy days 10 – 17 days. In sampling 2 (June – July), rainfall ranged from 15 – 134 mm, with the number of rainy days 7 – 10 days. In sampling 3, rainfall ranged from 65 – 199 mm, with the number of rainy days 8 – 15 days.<sup>54</sup>

However, the influence of the sampling station on the overall research data is more dominant than the sampling time, so all the independent variables studied (temperature, pH, nitrate concentration, phosphate, Cd, Cr, and Pb) did not have a significant effect on the dependent variables (genus number, abundance, and phytoplankton diversity index). Regression analysis to examine the pattern

of relationships between the independent variables (temperature, pH, concentration: nitrate, phosphate, Cd, Cr, and Pb) and the dependent variables (number of genera, abundance, and phytoplankton diversity index), showed that all independent variable's observation had no real contribution to the non-observational variables. The abundance of phytoplankton shows a marked difference between sampling times. The difference in phytoplankton abundance indicates that the distribution of phytoplankton is uneven in the waters. Plankton is an organism that has a patchy distribution pattern and a weak ability to move, so its distribution depends on the movement of water masses. Differences in the characteristics of physical and chemical parameters of waters such as fluctuations in currents, temperature, and nutrient concentrations can cause changes in phytoplankton abundance.<sup>55</sup> Slow-flowing rivers are more suitable for plankton life.<sup>56</sup> Temperature and nutrient concentration can control phytoplankton growth.<sup>42-44</sup> The dominant phytoplankton types found are the class Bacillariophyceae, then Chlorophyceae and Cyanophyceae (Appendix 3). This composition is similar to the results of Sharma's research, which found the class Bacillariophyceae dominates 95% of phytoplankton species, followed by Chlorophyceae (2.8%) and Cyanophyceae (1.6%), the rest from other phytoplankton classes.<sup>41</sup> Other research by Sharma obtained a similar composition with the dominance by classes Bacillariophyceae (36.3 - 80.5%), Chlorophyceae (18.1– 50.5%), Cyanophyceae (1.1–17.5%), and the rest of other phytoplankton classes.<sup>40</sup> Bacillariophyceae class or diatoms are a class of phytoplankton that easily adapt to various environmental conditions, including in polluted waters by multiplying mucus on their body surface.<sup>57,58</sup> Chlorophyceae are found in fresh and salt waters. The majority of them occur as submerged, freshwater plants being attached to submerged rocks, wood pieces, and similar other objects, but may also float on the surface of stagnant water forming green scums.<sup>59</sup> Cyanophyceae class will dominate waters that are slow-current, nutrient-rich (especially phosphorus), and warm temperatures.<sup>60</sup>

## Conclusion

The dominant phytoplankton class found in the Babon Rivers during the study consisted

of Bacillariophyceae, Chlorophyceae, and Cyanophyceae. The sampling station does not give rise to diversity against all independent variables (temperature, pH, nitrate, phosphate, Cd, Total Cr, Pb concentrations) and dependent variables (number of genera, abundance, and diversity index of phytoplankton). However, the sampling time causes diversity in nitrate, phosphate, Cd, Total Cr, and Pb concentrations. The sampling location effect was more dominant than sampling time, so the results showed that all independent variables did not contribute significantly to the dependent variables.

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### Conflict of Interest

The authors declare no conflict of interest.

### Data Availability Statement

The research paper includes all statistics information analyzed during the research work.

### Ethics Statement

The research did not involve human participants, animal subjects, or any material that requires ethical approval.

### Authors' Contributions

The research work was conducted by Dr. Haeruddin and Dr Frida Purwanti, senior lecturer in the Department of Aquatic Resources, Faculty of Fisheries and Marine Science, Diponegoro University, Central Java, Indonesia.

As young researchers, Arif Rahman and Kukuh Prakoso assisted in the Department of Aquatic Resources, Faculty of Fisheries and Marine Science, Diponegoro University.

Research plans, research contracts, leading data collection in the field, interpreting data, and writing articles are some of the tasks Dr. Haeruddin handles.

Dr. Frida Purwanti helped develop research strategies, interpret data, and write research papers.

Arif Rahman, M.Sc., prepares materials and tools for data collection, assists in data collection in the field, conducts data analysis, and helps write articles

Kukuh Prakoso, M.Sc., prepares correspondence for data collection permits, prepares transportation, assists in data collection in the field, conducts data analysis, and helps write articles.

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