Effect of Dissolved Nutrient Concentration (Nitrate and Orthophosphate) on Abundance of Chlorophyta in Penjalin Reservoir Brebes Regency

Novi Ariyanti[1], Carmudi[1], Christiani[1]
[1] Faculty of Biology, Jenderal Soedirman University, Purwokerto, Indonesia,
email: novikadharma@gmail.com

Abstract— This study aimed to find out the concentration of nitrate and orthophosphate in Penjalin Reservoir's water, to find out community structure (abundance, diversity and dominance) of Chlorophyta in Penjalin Reservoir's water and to confirm the effect of nitrate and orthophosphate concentration on Chlorophyta's abundance in Penjalin Reservoir's water. Survey method was performed at 6 station namely the center, the inlet and the outlet. Sampling was done 3 times with 4 weeks interval each. Water quality was analysed descriptively based on the criteria of quality standard in PP No. 82 tahun 2001 class III. The abundace of Chlorophyta was counted with volumetic method. The diversity was analysed with Shanon-Wienner's diversity index, while the dominance was analysed with Simpson's dominance index. The effect of nitrate and orthophosphate concentration on Chlorophyta's abundance was confirmed with correlation and regression analysis. Nitrate concentration was 0.348 mg.l-1 - 0.568 mg.l-1. Orthophosphate concentration was 0.006 mg.l-1 – 0.075 mg.l-1. Chlorophyta's abundance was 2.418 – 6.955 ind.l-1 with Tetraedron minimum as the most abundant species. Diversity index (H') value was 2,08 – 2,75 and dominance index (C) value was 0,08 – 0,21. The effect of nitrate and orthophosphate concentration on Chlorophyta's abundance was strong proven by correlation coefficient (r) value at 0,797 and determination coefficient value at 0,635 (63,50%). The regression equation was Y = 42,14 + 145 Nitrate + 1.280 Orthophosphate.

Key words—Nitrate, Orthophosphate, Abundance of Chlorophyta, Reservoir of Penjalin

INTRODUCTION

Chlorophyta is one of phytoplankton's division commonly found in fresh water and it act as primary producer [1]. Chlorophytas are able to produce organic materials from inorganic materials through photosynthesis, thus it can support the availability of zooplanktons and fishes' hatchlings [2]. Phytoplanktons' growth in water are significantly affected by phosphor and nitrogen. Phosphor and nitrogen exist in a very small amount in water hence they are often considered as limiting factor for phytoplanktons's growth [3].

Nitrogen are needed by organism in order to form protein, cell wall and body tissues [4]. In water, nitrogen can be found in its gas form N_2 which will transform into nitrate (NO_3), nitrate (NO_2), ammonia (NH_3), and ammonium (NH_4^+) [5]. Nitrate is one of nitrogen form that can be utilized by organism. It is resulted by decomposition of protein within dead remains [1]. Low nitrate containing water will has low abundance of phytoplankton whilst high nitrate content boost its abundance [6].

Phosphor are essential in protein synthesis, cell metabolism, and water productivity. In water, phosphor are available as inorganic molecules such as orthophosphate (PO_4^{3}), meta-phosphate ($P_3O_4^{3+}$), and polyphosphate ($P_3O_9^{3-}$) as well as organic molecules within organism's body [7]. Orthophosphate is the only form of phosphor that can be used directly by water organism, while polyphosphate must be hydrolyzed to form orthophosphate to enable its usage as phosphor source [8]. The availability of orthophosphate in water will affect the diversity and abundance of phytoplankton. Orthophosphate is used by phytoplankton to grow and it is absorbed optimally when the concentration is less than 1 $mg.l^{-1}$ [9]. The present study was undertaken with the following objectives:

1. To find out the concentration of nitrate and orthophosphate in Penjalin Reservoir's water, Brebes Regency.
2. To find out the community structure (abundance, diversity and dominance) of Chlorophyta in Penjalin Reservoir, Brebes Regency
3. To understand the effect of nitrate and orthophosphate concentration on the abundance of Chlorophyta in Penjalin Reservoir, Brebes regency

METHODS

a. Research Location

The Research located in Penjalin Reservoir, Winduaji Village, Paguyangan District, Brebes, Central Java at coordinate 6°44'56" SL - 7°20'51,48" SL and 108°41'37" EL - 109°11'29" EL.

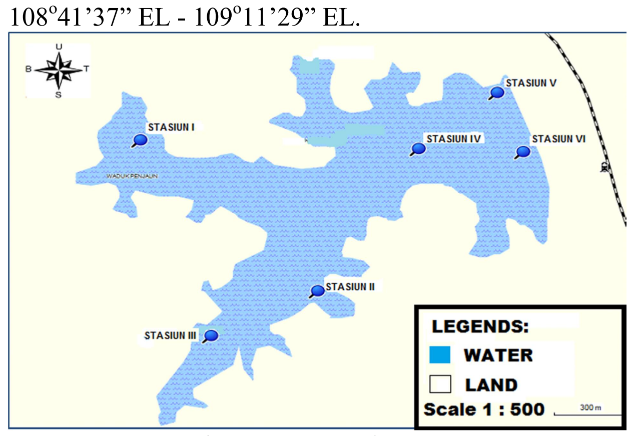

Figure 1. Research Sites in Penjalin Reservoir

Explanation :
Station 1: Center of the reservoir
Station 2: Inlet from Garung River
Station 3: Inlet from Penjalin River
Station 4: Inlet from Soka River
Station 5: Outlet
Station 6: Dock Area

Measurement of physical and chemical parameters of waters is take place in the research location, in the Laboratory of Environmental and Aquatic Laboratory, Faculty of Biology, Jenderal Soedirman University. Identification and analysis of plankton were performed at Aquatic Biology Laboratory of Biology Faculty Universitas Jenderal Soedirman Purwokerto. This research was conducted in June-August 2015.

This research used survey method. Chlorophyta's abundance was set as dependent variable while nitrate and orthophosphate concentration as independent variable. Main parameters of this research were the number of Chlorophyta's species and individual as well as nitrate and orthophosphate concentration. As supportive parameters, physical and chemical properties of water were measured included temperature, light penetration, depth, pH, Dissolved Oxygen (DO) concentration, free CO_2 concentration, Biochemival Oxygen Demand (BOD), Total Suspended Solid (TSS), Total Dissolved Solid (TDS), Total Nitrogen concentration and Total Phosphor concentration.

b. Measurements of Chlorophyta Samples

Chlorophya Sampling was done by filtering 100 liters of water reservoirs using a plankton-net No. 25, and then transferred into the sample bottles that had been labeled, then add 3 - 4 drops of lugol and formalin concentration from 40% to 4%. Formalin can also be obtained by using the following formula:

$$C_1 . V_1 = C_2 . V_2$$

Explanation :
C_1 = Desired concentration of formalin
C_2 = Available concentration of formalin
V_1 = Volume of water in the bottle
V_2 = Volume of formalin needed

Chlorophyta were found then identified using a book of Sachlan [16] and Davis [4], as well as its abundance is calculated by a volumetry Method [2]:

$$N = \frac{Vr \times n}{Vo \times Vs}$$

Explanation :
n : Observed imdividual
N : Individual.liter
Vo : One drop volume (0,05ml)
Vr : The volume filtered out (100ml)
Vs : The volume filtered (100000 ml)

c. Measurements of Physical and Chemical Parameters

Measurements of physical and chemical parameters that shown in Table 1.

Table 1. Measurements of physical and chemical parameters

No.	Parameters	Methods
1	Air and Water Temperature	Pemuaian [10]
2	Depth	Depht sounder
3	Light Penetration	Secchi [11]
4	Total Suspended Solid (TSS)	Gravimetri [10]
5	Total Disolved Solids (TDS)	TDS meter
6	Orthophosphate (PO_4)	Spekofotometri [12]
7	Nitrate (NO_3)	UV Spectrofotometric [13]
8	pH	[12]
9	free CO_2 concentration	Titrimetri [11]
10	Dissolved Oxygen (DO)	Winkler [14]
11	Biochemical Oxygen Demand (BOD)	Winkler [15]
12	Total Nitrogen	Mikrokjeldahl [13]
13	Total Phosphor	Asam askorbik [13]

d. Analysis Methods

1. Water quality was analysed descriptively based on the criteria of quality standard in PP No. 82 tahun 2001 class III.
2. Chlorophyta's community structure (abundance, diversity and dominance). The number of Chlorophyta's individual was analysed with descriptive method to see its abundance. Chlorophyta's diversity was analysed with Shannon-Wienner's diversity index in [16] using the following formula:

$$H' = -\sum_{1=1} pi \ln pi$$

Explanation:
H' : Shannon-Wienner's diversity index
Pi : ni/N
ni : number of individual species i
N : number of total individual

Criteria of Shannon-Wienner's diversity index value in [8]:
$H' < 1$: low diversity
$1 < H' < 3$: moderate diversity
$H' > 3$: high diversity

Simpson's dominance index is to determine if there is dominating species in the water. Simpson's equation in [1] is written as follow:

$$C = \sum(pi)^2 = \sum\left(\frac{ni}{N}\right)^2$$

Explanation:
C : Simpson's dominance index
ni : number of individual species i
N : number of total indidual

Criteria of Simpson's dominance value in [17]:
$0.5 < C < 1$: dominating species exists
$0 < C < 0.5$: dominating species does not exist

3. The relation between nitrate and orthophosphate and Chlorophyta's abundance in Penjalin Reservoir was analyzed with regression-corelation in SPSS.

RESULTS

a. Nitrate and Orthophosphate Concentration and other Physical-Chemical Parameter in Penjalin Reservoir, Brebes Regency

Based on quality standard in [18] of class III's water quality, all parameters that were measured in Penjalin Reservoir's water were good for growth and development of aquatic organism since they didn't exceed the determined value (Table 2). Nitrate measurement result was 0.348 mg/l – 0.568 mg/l. Orthophosphate measurement result was 0.006 mg/l – 0.075 mg/l (Table 2).

Table 2. Result of Physical and Chemical Parameter Measurement in Penjalin Reservoir's Water

Parameters	Unit	Station						Avarage	Std. Deviation	Water quality standard class III
		I	II	III	IV	V	VI			
Nitrate	$mg.l^{-1}$	0,367	0,348	0,460	0,414	0,568	0,506	0,448	0,084	<20
Orthophosphate	$mg.l^{-1}$	0,006	0,040	0,038	0,007	0,012	0,075	0,03	0,027	<1
Water temperature	°C	26	25,83	27	26,67	26,33	26	26,3	0,45	-
Air temperature	°C	25	24,67	25,33	24,33	24,67	25,33	24,89	0,4	-
Light Penetration	m	1,41	1,29	6,61	1,53	1,29	1,29	2,24	2,14	-
Depht	m	4,1	3,27	2,9	8,87	5,6	4,43	4,86	2,18	-
DO	$mg.l^{-1}$	5,87	5,77	7,1	6,97	6,93	7,03	6,61	0,62	3
pH		6	6	6	6	6	6	6	0	6-9
BOD	$mg.l^{-1}$	2,35	1,78	1,09	1,75	3,09	2,87	2,15	0,76	6
Free CO_2	$mg.l^{-1}$	3,19	1,58	2,24	3,3	2,68	3,15	2,69	0,67	-
TSS	$mg.l^{-1}$	3,35	3,33	2,99	3,36	3,69	3,32	3,34	0,22	<400
TDS	$mg.l^{-1}l$	50,67	52,67	51	50,67	51,33	32,67	48,17	7,63	<1000
Total N	$mg.l^{-1}$	4,18	4,37	4,09	4,65	5,07	4,16	4,42	0,38	20
Total P	$mg.l^{-1}$	0,073	0,392	0,214	0,018	0,046	0,207	0,16	0,14	1

Nitrogen source in Penjalin Reservoir's water was estimated to coming from domestic waste and agriculture activity that was brought by water of Kali garung, Kali Penjalin and Kali Soka. Water Catchment Area (WCA) is an area within a topographic border (hill surface) that absorbs rain water which then flows to river branches and gathers at a main river to end up at sea [19]. Agriculture activities around a reservoir contributes to nitrogen content in water body through the use of fertilizer. The excess fertilizer will be washed off by rain into river or other water body. Urea is commonly used fertilizer. According to [20] nitrogen content within urea fertilizer is 46%.

Domestic waste or household waste consists of any kind of waste produced by the activity related to sanitation, cooking, and washing. [21], stated that human's feces contained 5-7% N, while human's urine contained 15-19% N. Activities on WCA affected nitrate concentration at station V (outlet area) by 0.058 $mg.l^{-1}$, relatively higher than any other stations (Table 2). It was because there was villager residence around the outlet area that disposed their domestic waste to the water. In addition, outlet area was the only exit way for the water thus all wastes in reservoir water gathered at this point.

Highest orthophosphate concentration was at station VII (dock area) with 0.075 $mg.l^{-1}$ (Table 2). The availability of phosphate in Penjalin Reservoir's water was estimated to coming from fertilizer residual and detergent within domestic waste. [22] stated that commonly used P containing fertilizer was SP 36 in accordance to its availability in the market. [23], stated that P concentration in SP 36 fertilizer was 36%.

Orthophosphate originated from domestic waste is mainly from detergent and human's excretion-urine and feces. As main source of phosphor, detergent contributed to water eutrophication by 7-12% [24]. Detergent in common contains materials that can be categorized as surface-active agents or surfactant, builders or building materials and additive substances or additional materials [25].

b. Community Structure of Chlorophyta in Penjalin Reservoir, Brebes Regency

Community structure of Chlorophyta we observed were their abundance, diversity and dominance. The result given was that 49 species of Chlorophyta from 30 genera existed in Penjalin Reservoir's water (Table 3). Total abundance of Chlorophyta in Penjalin Reservoir ranged between 2.418 – 6.995 $ind.l^{-1}$. Presented in table 3, *Tetraedon minimum* possessed the highest relative abundance with 18,28% followed by *Zinegma sp.* 17.46% and *Staurastrum tetracerum* 7.67%.

Table 3. Chlorophyta's Abundance (ind.l^{-1}) in Penjalin Reservoir's Water

No	Name of Species	Abundance of Chlorohyta (Ind.l^{-1}) Station I	II	III	IV	V	VI	Abundance average (Ind.l^{-1})	RA %
1	*Ankistrodesmus falcatus*	118	21	11	43	107	183	484	1,95
2	*A. spiralis*	-	11	-	301	21	-	333	1,34
3	*Asterococcus* sp.	-	-	-	-	86	64	150	0,61
4	*A. superbus*	43	215	140	21	43	64	527	2,12
5	*Botryococcus* braunii	107	344	387	301	161	442	1742	7,02
6	*Chlamydocapsa* sp.	-	-	-	-	193	409	602	2,43
7	*Chlamydomonas* sp.	97	129	-	43	43	193	505	2,04
8	*Chlorococcum humicola*	11	-	-	54	-	-	64	0,26
9	*Closterium porrectum*	-	21	-	-	-	-	21	0,09
10	*C. astroideum*	-	64	11	11	-	-	86	0,35
11	*Coelastrum reticulatum*	-	21	32	11	-	11	75	0,30
12	*C. sphaericum*	-	-	11	11	21	-	43	0,17
13	*Cosmarium depressum*	172	236	97	64	408	247	1225	4,94
14	*C. phaseolus*	355	226	-	193	204	279	1257	5,07
15	*C. quadratum*	-		32	-	-	-	32	0,13
16	*Eudorina* sp.	-	204	-	-	-	43	247	1
17	*Gloeocystis* sp.	-	64	-	43	86	150	344	1,39
18	*Golenkinia radiate*	140	129	76	21	54	32	452	1,82
19	*Gonium pectoral*	-	-	-	64	43	-	107	0,43
20	*Haematococcus* sp.	21	-	-	32	-	21	75	0,30
21	*Micrasterias denticulate*	21	32	-	-	-	-	54	0,22
22	*Microspsora* sp.	21	76	11	183	-	21	52	1,26
23	*Oocystis* sp.	-	-	-	-	-	43	7	0,17
24	*Mougeotia* sp.	43	54	21	344	172	-	106	2,56
25	*Pandorina morum*	290	258	107	247	172	333	235	5,68
26	*Pediastrum boryanum*	32		21	76	-	-	22	0,52
27	*P. duplex*	11	43	43	-	-	-	16	0,39
28	*P. simplex*	43	75	97	32	43	43	56	1,34
29	*P. tetras*	-	-	-	-	-	11	2	0,04
30	*Polyedriopsis spinulosa*	21	-	-	54	108	64	41	1
31	*Rhizoclonium* sp.	11	-	-	-	-	-	2	0,04
32	*Scenedesmus acuminatus*	-	32	-	11	-	11	9	0,22
33	*S. bijugatus*	32	129	-	21	21	161	61	1,47
34	*S. dimorphus*	-	-	21	-	32	-	9	0,22
35	*S. armatus*	11	86	43	21	-	118	47	1,13
36	*S. subspicatus*	-	21	-	-	86	21	21	0,52
37	*S. quadricauda*	11	32	-	32	-	11	14	0,35
38	*Staurastrum anatinum*	21	-	-	-	21	-	7	0,17
39	*S. chaetoceros*	-	-	-	21	11	-	5	0,13
40	*S. grallatorium*	32	-	21	-	-	-	9	0,22
41	*S. teliferum*	11	-	-	-	-	-	2	0,04
42	*S. longispinum*	-	-	11	-	-	-	2	0,04
43	*S. tetracerum*	387	527	107	236	269	376	317	7,67
44	*Staurodesmus convergens*	11	43	43	11	-	-	18	0,43
45	*Tetraedron minimum*	699	838	-	731	827	1440	756	18,28
46	*Volvox* sp.	54	75	-	-			21	0,52
47	*Zygnema* sp.	54	591	1010	849	408	1418	722	17,46
48	*Z. pectinatum*	-	11	43	-	172	666	149	3,60
49	*Zygogonium* sp.	-	-	21	43		76	23	0,57
	Total of individual per station	**2.880**	**4.611**	**2.418**	**4.127**	**3.816**	**6.955**	**4.134**	**100**
	Total of species per station	**29**	**30**	**24**	**31**	**26**	**30**		

The stability of a community is reflected by its dominance index's and diversity index's value. The value of dominance index and diversity index of Chlorophyta in Penjalin Reservoir is presented in table 4.

Table 4. Value of Diversity Index (H′) and Dominance Index (C) of Chlorophyta in Penjalin Reservoir's Water

Station	Diversity index (H′) value	Dominance index (C) value	Information
I	2,6	0,11	moderate diversity and dominating species does not exist
II	2,75	0,08	moderate diversity and dominating species does not exist
III	2,08	0,21	moderate diversity and dominating species does not exist
IV	2,58	0,1	moderate diversity and dominating species does not exist
V	2,7	0,09	moderate diversity and dominating species does not exist
VI	2,58	0,11	moderate diversity and dominating species does not exist

Shannon-Wienner's diversity index (H′) value of each station ranged between 2.08 – 2.75 (Table 4). Based on the criteria of diversity index value in [], Chlorophyta's diversity in Penjalin Reservoir's water was moderate (1< H′< 3). Simpson's dominance index (C) value of each station ranged between 0.08 – 0.21 (Table 4). This value indicated that there was no dominating species. According to [10], dominance index value is 0 – 1, if the value approaches 0 (< 0.5), there is no species that dominates the other. In the other hand, if the value approaches 1 (> 0.5), the species is dominating the other. Such value given in the result indicated that there was no species extremely dominating the other, furthermore the environment condition was stable so there was no ecological pressure towards biota in this environment.

c. Relation between nitrate and orthophosphate concentration and Chlorophyta's Abundance in Penjalin Reservoir' Water, Brebes Regency

The result of correlation analysis between Chlorophyta's abundance and each of nutrients (nitrate and orthophosphate) given the coefficient at 0.38, 0.758 and 0.797, hence the relation of each value could be defined as low, high and high respectively (Table 5). According to Sugiono (2004), if correlation value (r) is 0.20 – 0.399, it means that relation between variables is low, and if correlation value (r) was 0.60 – 0.799, it means that relation between variables is high.

Table 5. Result of Regression-Correlation Analysis of Nitrate and Orthophosphate Concentration with Chlorophyta's Abundance

Parameters	Abundance of Chlorophyta: Correlation coefficient (r)	Level of relationships	Determination coefficient (R^2)	regression equation
K-Nitrate	0,38	Low	15,0%	-
K- orthophosphate	0,758	Strong	57,4%	Y= 104 + 1.367 orthophosphate
K- nitrate and orthophosphate	0,797	Strong	63,50%	Y= 42,1 + 145 Nitrate + 1.280 orthophosphate

*K: Abundance of Chlorophyta

The result of simple regression analysis to determine the relation between orthophosphate and Chlorophyta's abundance was following the equation Y= 104 + 1.367 Orthophosphate. The equation explained that in the absence of orthophosphate, Chlorophyta's abundance was 104 ind.l^{-1}. The increasing of orthophosphate concentration by 0.1 mg.l^{-1} would increase Chlorophyta's abundance by 1367 ind.l^{-1}.

The result of multiple regression analysis to determine the relation between nitrate and orthophosphate simultaneously towards Chlorophyta's abundance was following the equation Y = 42.1 + 145 Nitrate + 1280 Orthophosphate. The equation explained that in the absence of nitrate and orthophosphate, Chlorophyta's abundance was 42.1 ind.$l^{-1.}$ The increasing of nitrate concentration by 0.1 mg.l^{-1} would increase Chlorophyta's abundance by 145 ind.l^{-1}. The increasing of orthophosphate concentration by 0.1 mg.l^{-1} would increase Chlorophyta's abundance by 1280 ind.l^{-1}.

Relation strength between nitrate and orthophosphate simultaneously was shown by determination coefficient (R^2) at 63.50%, which meant Chlorophyta's abundance was affected by nitrate and orthophosphate altogether at 63.50%, and the remaining 36.50% was affected by other factors including physical and chemical factors of water namely pH, temperature, depth, light penetration, TSS, TDS, dissolved O_2, free CO_2, BOD, Total N and Total P.

CONCLUSION

Based on the results and discussion we concluded:

1. Concentration of nitrate and orthophosphate in Penjalin Reservoir was 0,348 mg.l^{-1} – 0,568 mg.l^{-1} and 0,006 mg.l^{-1} – 0,075 mg.l^{-1} respectively.
2. Community structure of Chlorophyta in Penjalin Reservoir had total abundance of 2418 – 6955 ind.l^{-1} , moderate diversity (H = 2,08 – 2,75), and no dominating secies (C = 0,08 – 0,21).
3. Chlorophyta abundance was highly affected by nitrate and orthophosphate with correlation coefficient r = 0,797, determination coefficient R^2 = 63,50% and the equation was Y= 42,1+145 nitrate + 1.280 orthophosphate.

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