NO_3 and H_2S vertical stratification based on mangrove zone at Bedono Village, Demak, Central Java

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Abstract. Demak Regency has a mangrove ecosystem with an area of 2089.45 ha. Bedono Village has a mangrove ecosystem of 197.19 ha. Mangrove areas produce organic matter from decomposition and become a supplier of nutrients to their environment. The process involves oxygen and bacteria. If it occurs aerobically, nitrate compounds are formed, while H_2S compounds are formed anaerobically. This study examines the potential for nutrient release differences between zoning and sediment depths. The method used in this study is the explanatory method. The sampling was carried out randomly in 3 zones with three sampling points each. The sampling of sediments and measurements were based on three levels of depth: 0-20 cm, 20-40 cm, and 40-60 cm. The variables measured were nitrate and sediment H_2S. The results show that the release of nitrates ranged between 0.02 mg 100 g^{-1} and 1.65 mg 100 g^{-1}, and H_2S between 0.02 mg L^{-1} and 0.07 mg L^{-1}. Based on the analysis of nitrate content, there was a significant difference in nitrate in zoning and depth. At the same time, the H_2S had significant differences between depths at the study site. The release of nutrients at the study site is more likely to be influenced by the decomposition process of organic matter and environmental factors such as sea and river water input and substrate type, mangrove density, the abundance of organisms and anthropogenic activities.
Key Words: bacteria, H_2S, mangroves, nitrate, organic matter.

Introduction. Bedono village mangrove forest area has a decreased quality. Of the 5207 ha of mangrove forests in Demak, 62 ha are in degraded conditions, according to the 2019 forecast data from the Department of Marine Affairs and Fisheries of Demak Regency. This is caused by several factors, including waves, the conversion of natural resources, and the impact of climate change. These conditions affect the productivity of coastal or estuarine ecosystems that support the availability of fish resources (Widhitama et al 2016).

Mangrove ecosystems are areas with high primary productivity. Bacteria can use the content of existing organic matter to accelerate the process of decomposition in the water. The essential elements produced are nitrate and phosphate, which can support the growth and development of mangroves. The availability of nutrients, especially N, affects the decomposition rate of organic material on land and in mangrove forests (Hayes et al 2017). Decomposition is an essential process in ecological functions (Saibi & Tolangara 2017), maintaining maintains soil fertility in mangrove forests by providing essential elements and food sources for various local biota. This is reinforced by Schaduw (2018), who noted that necessary nutrients, such as nitrate, are indicators of water fertility and arise from the complete oxidation process of nitrogen compounds in the body of the water. Adding organic matter into the soil increases the activity of microorganisms that degrade organic matter, especially the abundance of nitrogen and carbon microbes. Increasing this activity ultimately increases nitrogen availability through mineralization (Wijanarko et al 2012).

If the decomposition process occurs continuously, it can cause the formation of hydrogen sulfide compounds (H_2S). Hydrogen that is not ionized is toxic to aquatic life. Toxic H_2S is formed due to the activity of chemoautotrophic bacteria that use sulfate from the process of reshuffling organic matter as a source of energy. H_2S can also regulate critical metabolism in plants. H_2S can mediate an increase in plant tolerance to salinity, drought, heavy metal pollution, and high-temperature stress (Vojtovič et al 2020). The process of releasing nutrients in mangrove sediments needs to be examined because nutrients are essential to support the growth and development of the potential resources of mangrove ecosystems (Handayani et al 2016).

The present study aims to examine the potential for nutrient release differences between zoning and sediment depths and might be used as a reference for the people of Bedono Village and the Demak Regency Government in preserving the ecological environment, managing and utilizing the Potential of Mangrove Ecosystems in an integrated and sustainable manner.

Material and Method

Description of the study sites. This study was conducted at Bedono Village, located in Demak Regency, on the northern coast of mid-Java. The village of Bedono lies within latitudes 6º54'38.6"-6º55'54.4" south and longitude 110º28'39.6"-110º30'22.8" east. This region has experienced massive devastation with an average erosion rate of -25 m year^{-1}, while the shoreline movement was -592 m (Muskananfola et al 2020a). It is a low-lying region with an elevation of less than 2% and a height of 0-5 m above sea level, dominated by silt and clay sediments (Muskananfola et al 2020b). There are mangrove areas covering some portions of the shore, but also dense mangrove ecosystems dominated by *Avicennia marina*.

Field sampling. Sediment samples were collected in March 2022 at three zones in the mangrove areas of Bedono Demak (Figure 1). Three replicate samples were collected at each zone within the three zones. A sampling of sediments based on three depth levels, 0-20 cm, 20-40 cm, and 40-60 cm, were collected with a modified pipe of 5 cm diameter and 60 cm length.

The main research variables are nitrate and H_2S from mangrove areas. Bacteria, oxygen, and organic matter are factors in the degradation process in each zone and explanatory variables such as pH, temperature, sediment texture, salinity and sediment carbon were also determined.

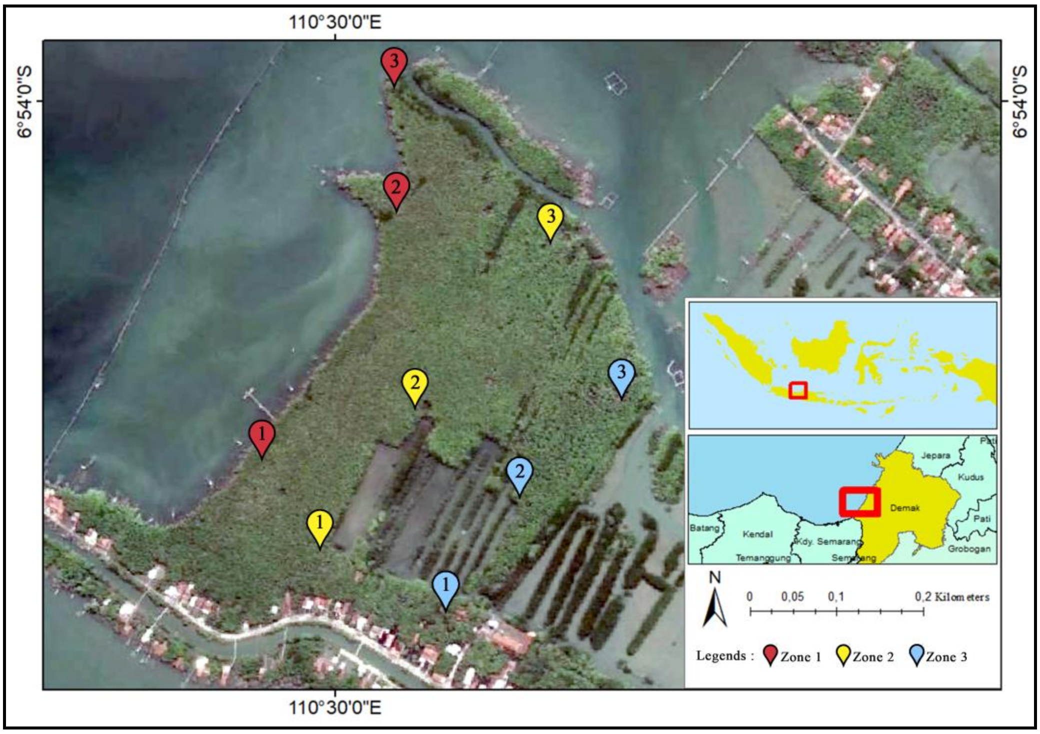

Figure 1. The location of mangrove areas at Bedono, Demak, Indonesia.

Measurements of environmental data were carried out *in situ* at the same time the sediment samples were collected. Dissolved oxygen (DO), pH, temperature, and salinity were measured using the Water Quality Checker U-53G Series at each sampling site. The calculation and identification of mangrove density refers to the Decree of the Minister of the Environment No. 201 of 2004.

Data analysis. Nitrate sediment was analyzed using the UV-VIS spectrophotometer at wavelengths 220 nm and 275 nm (Yulianti et al 2022).

H_2S sediment was analyzed using the UV-VIS spectrophotometer at a wavelength of 644 nm (Brahmi & Abderafi 2021) using the following formula:

$$S^{2-}(\text{mg L}^{-1}) = \frac{A}{(\text{slope} \times V)} \times \frac{V2}{V1} \times f$$

Where: A - absorbance of the test sample; V - volume of the test sample (mL); V1 - the final volume of the test sample (mL); V2 - initial volume of the test sample (mL); f - dilution factor.

The sediment grain size composition was quantified using the sieve analysis method (Buchanan 1984). Organic matter in the sediment was analyzed using the loss of ignition method (Heiri et al 2001), with the following formula:

$$\%\ BOT = \frac{(Wt - C) - (Wa - C)}{Wt - C} \times 100$$

Where: Wa - total crucible and sample weight before being dried (g); C - empty crucible weight (g); Wt - total crucible weight and sample after drying (g); organic matter conversion = % organic matter x 1.724.

The total bacteria test was based on the total plate count (TPC) using three methods: pour plate, spread plate, and drop plate. Total Plate Count includes all colonies growing on nutrient agar (NA) media. Total bacteria were counted on the Petri cup. Afterwards, the amount obtained was multiplied by the dilution, using the following formula:

$$N = \frac{100}{\text{Volume} \times \text{Inoculation}} \times \sum \text{Colony} \times \text{Dilution Factor}$$

Where: N - abundance of bacteria (CFU mL^{-1}); CFU - colony forming unit.

Mangrove density was calculated by identifying mangrove species, the number of individual species of mangroves and tree diameter (trees at chest height - ±130 cm - diameter >10 cm or rod >31 cm; stakes for stem diameter <10 cm and height of plants >1.5 m; seedling for plants with a height <1.5 m) in a quadrant with a size of 10x10 m, by using the following formula:

$$Di = \frac{Ni}{A}$$

Where: Di - density- i (ind m^{-2}); Ni - total number of individuals of the species -i (ind); A - total area of sampling (m^2).

Data analysis was carried out by using a parametric test, a Two-Way ANOVA, with two factors: zoning and depth. In this case, the zoning used as the first factor of research is the advanced zone of the mangrove area, the middle mangrove area and the inner area. The second factor is the depth of pore water in 3 levels, namely 0-20, 20-40, and 40-60 cm. The test was carried out on the main variables, namely nitrate and H_2S. The Principal Component Analysis (PCA) was applied to find the closeness between variables during this research. It can be used to explain the differences in the main variables tested in the study area. Multiple linear regression analysis was applied to determine the factors that affect nitrate and H_2S, as well as to see the relationships between nitrate and H_2S with other variables.

Results and Discussion

Environmental variables. Environmental values are presented in Table 1. DO and pH decreased with increasing depth, where the highest DO value at a depth of 0-20 cm was 0.67 mg L^{-1}, and the highest pH was 7. The highest temperature and salinity were at a depth of 0-20 cm, with 30.2°C and 48‰, respectively. However, the measured temperature and salinity show fluctuations between sediment depths. The sediment textures were dominated by silt ranging from 84-97%.

Organic matter. Organic matter values are presented in Table 2. Organic matter in the sediment had the highest average at a depth of 0-20 cm in zone II, with 21.42±3.01%, while the lowest was 17.41±0.4% at a depth of 40-60 cm in zone III.

Organic matter was then analyzed to test whether there are differences among stations and depths (Table 3). There were differences between research stations and between depth levels ($p<0.05$). The highest levels of organic matter were found in the middle zone (zone II), while zones I and III did not present differences. Meanwhile, based on depth, the 0-20 cm layer (surface) had the highest organic matter content, the content decreasing with increasing depth.

The organic carbon content is presented in Table 4. The organic carbon has the highest average at a depth of 0-20 cm in zone II, with 12.43±1.75%, while the lowest was 10.10±0.24% at a depth of 40-60 cm in zone III.

Nitrate. Nitrate values presented in Table 5 have the highest average at a depth of 0-20 cm in zone II, 1.39±0.34 mg 100 g^{-1}, and the lowest is 0.17±0.13 mg 100 g^{-1}, at a depth of 40-60 cm in zone III.

There are differences in nitrate content between research stations and between depth levels ($p<0.05$) (Table 6). Furthermore, the highest nitrate level was found in the front zone (zone I), followed by zone II and the lowest levels were found in zone III. Meanwhile, based on its depth, the 0-20 cm layer (surface) has the highest nitrate, and the lower is followed by increasing depth.

H_2S. H_2S values presented in Table 7 had the highest average at a depth of 40-60 cm in zone I and II, of 0.05±0.02 mg L^{-1} and the lowest, 0.02 mg L^{-1}, at a depth of 0-20 cm in zones II and III.

There are differences between depth levels ($p<0.05$) (Table 8) in H_2S content. Based on the differences among stations, the levels of H_2S found in zones I, II and III are similar. Based on the depth, the highest H_2S content was in the 40-60 cm, being lower towards the surface.

Total bacteria. Total bacteria values are presented in Table 9, with the highest average at a depth of 0-20 cm in zone II, of $236x10^5$±75.29 CFU mL^{-1} and the lowest at $8.7x10^5$±10.75 CFU mL^{-1} at a depth of 40-60 cm in zone III.

There are differences between research stations and between depth levels ($p<0.05$) (Table 10). Furthermore, the highest bacterial levels are found in zone II, while zones I and III do not present significant differences. Meanwhile, the 0-20 cm layer has the highest bacterial content, which decreases with increasing sediment depth.

Table 1

Environmental values of the study area

Zone	*Depth (cm)*	*Dissolved oxygen (mg L^{-1})*			*pH*			*Temperature (°C)*			*Salinity (‰)*			*Silt (%)*	*Clay (%)*	*Sand (%)*
		1	*2*	*3*	*1*	*2*	*3*	*1*	*2*	*3*	*1*	*2*	*3*			
	0-20	0.56	0.42	0.20	6.9	6.8	6.2	30.3	30.2	30.1	28	36	37	84	12.5	3.5
I	20-40	0.47	0.35	0.09	6.3	6.4	5.8	29.8	29.6	29.6	28	30	32	86	10.8	3.2
	40-60	0.43	0.25	0.06	5.6	5.5	5.4	29.6	29.5	29.3	36	32	47	97	2.72	0.28
	0-20	0.67	0.42	0.11	7	6.9	6.8	29.6	29.2	29.5	36	30	48	88	9.10	2.9
II	20-40	0.59	0.60	0.18	6.8	6.2	6.5	28.5	28	27.9	30	38	40	89	8.27	2.73
	40-60	0.63	0.47	0.13	5.6	5.7	5.8	29.6	29.3	29.1	25	23	27	92	5.13	2.87
	0-20	0.58	0.21	0.05	7	6.9	6.9	27.8	27.5	28.1	25	25	28	94	4.7	1.3
III	20-40	0.51	0.22	0.16	6.4	6.1	6	29.7	29.4	28.9	27	28	34	94	4.9	1.1
	40-60	0.47	0.28	0.12	5.6	5.5	5.8	28.8	28.7	28.5	30	25	30	95	3.04	1.96

Table 2

Organic matter values of the study area

Zone	*Depth (cm)*	*Replicate samples (%)*			*Average (Mean±SD)*
		1	*2*	*3*	
I	0-20	19.53	19.93	19.81	19.76±0.21
	20-40	18.65	19.77	18.35	18.93±0.75
	40-60	16.39	19.62	17.52	17.84±1.64
II	0-20	24.48	21.32	18.47	21.42±3.01
	20-40	20.16	20.76	18.33	19.91±1.27
	40-60	20.03	19.41	17.78	19.07±1.16
III	0-20	18.49	19.18	18.45	18.71±0.41
	20-40	17.93	18.45	18.35	18.25±0.28
	40-60	17.35	17.84	17.04	17.41±0.40

Table 3

Tests of between-subjects effects

Source	*Type III sum of squares*	*df*	*Mean square*	*F*	*Sig.*
Zone	17.726	2	8.863	5.084	.018
Depth	15.493	2	7.746	4.443	.027

Table 4

Organic carbon values of the study area

Zone	*Depth (cm)*	*Replicate samples (%)*			*Average (Mean±SD)*
		1	2	3	
I	0-20	11.33	11.56	11.49	11.46±0.12
	20-40	10.82	11.47	10.64	10.98±0.44
	40-60	9.50	11.38	10.16	10.35±0.95
II	0-20	14.20	12.37	10.71	12.43±1.75
	20-40	11.69	12.04	10.63	11.45±0.73
	40-60	11.62	11.26	10.31	11.06±0.68
III	0-20	10.73	11.13	10.70	10.85±0.24
	20-40	10.40	10.70	10.65	10.58±0.16
	40-60	10.06	10.35	9.88	10.10±0.24

Table 5

Nitrate values of the study area

Zone	*Depth (cm)*	*Replicate samples (mg 100 g^{-1})*			*Average (Mean±SD)*
		1	2	3	
I	0-20	1.43	1.01	1.65	1.36±0.33
	20-40	1.21	0.70	0.99	0.97±0.26
	40-60	0.51	0.59	0.68	0.59±0.09
II	0-20	1.63	1.54	1.00	1.39±0.34
	20-40	1.33	1.12	0.74	1.06±0.30
	40-60	0.35	0.11	0.55	0.34±0.22
III	0-20	0.98	0.75	0.87	0.87±0.12
	20-40	0.63	0.51	0.28	0.47±0.18
	40-60	0.02	0.24	0.25	0.17±0.13

Table 6

Tests of between-subjects effects

Source	*Type III sum of squares*	*df*	*Mean square*	*F*	*Sig.*
Zone	1.216	2	.608	11.064	.001
Depth	3.206	2	1.603	29.181	.000

Table 7

H_2S values of the study area

Zone	*Depth (cm)*	*Replicate samples (mg L^{-1})*			*Average (Mean±SD)*
		1	*2*	*3*	
I	0-20	0.03	0.03	0.02	0.03±0.01
	20-40	0.04	0.04	0.02	0.03±0.01
	40-60	0.05	0.07	0.03	0.05±0.02
II	0-20	0.02	0.02	0.02	0.02±0.00
	20-40	0.03	0.02	0.03	0.03±0.01
	40-60	0.04	0.06	0.06	0.05±0.01
III	0-20	0.02	0.02	0.02	0.02±0.00
	20-40	0.02	0.03	0.03	0.03±0.01
	40-60	0.05	0.04	0.03	0.04±0.01

Table 8

Tests of between-subjects effects

Source	*Type III sum of squares*	*df*	*Mean square*	*F*	*Sig.*
Zone	.000	2	9.676E-5	.909	.421
Depth	.003	2	.002	14.463	.000

Table 9

Total bacteria values of the study area

Zone	*Depth (cm)*	*Replicate samples (10^5 CFU mL^{-1})*			*Average (Mean±SD)*
		1	2	3	
I	0-20	93	112	122	109±14.73
	20-40	18	74	34	42±28.84
	40-60	3.5	19	5	9±8.55
II	0-20	170	318	220	236±75.29
	20-40	70	76	193	113±69.35
	40-60	2	3	22	9±11.27
III	0-20	197	143	56	132±71.14
	20-40	7.4	8	26	13.8±10.57
	40-60	21.1	3	2	8.7±10.75

Table 10

Tests of between-subjects effects

Source	*Type III sum of squares*	*df*	*Mean square*	*F*	*Sig.*
Zone	268607962962963.280	2	134303981481481.640	7.104	.005
Depth	1059174429629629.000	2	529587214814814.500	28.013	.000

Mangrove density. The density of mangroves in the study area is presented in Table 11. Mangroves found at the location are *Rhizophora mucronata* and *Avicennia marina*. The highest density was found in zone II, with *A. marina* having more individuals.

Table 11

Mangrove density of the study area

Zone	*Species*	*Individuals*	*Density (ind ha^{-1})*	*Decree of the State Minister for Environment Number 201 of 2004*
I	*Avicennia marina*	159	5300	>1500 (high)
	Rhizophora mucronata	22	1100	>1000-<1500 (medium)
II	*Avicennia marina*	176	5867	>1500 (high)
III	*Avicennia marina*	105	3500	>1500 (high)
	Rhizophora mucronata	25	1250	>1000- <1500 (medium)

The variables were extracted using PCA and categorized into three components, as presented in Table 12. The first component can explain the relationship between variables by 49.55% of the total diversity. The analysis results show a relatively clear and straightforward correlation among depth, nitrate, H_2S, organic matter, DO and bacteria.

Table 12

Rotated component matrix results

Rotated component matrix				
No	*Variables*	*Component*		
		1	*2*	*3*
1	Zone	-.151	-.156	.944
2	Depth	-.866	.365	-.089
3	Replicate	-.268	-.851	-.253
4	Nitrate	.898	-.072	-.326
5	H_2S	-.690	.483	-.311
6	Organic matter	.755	.290	-.135
7	Dissolved oxygen	.909	-.219	.042
8	Total bacteria	.664	.666	.210

Factors that affect nitrate and H_2S were determined using the multiple linear regression analysis. The relationship of nitrate with other variables can be seen in the regression model presented in Table 13.

Table 13

Multiple linear regression analysis between nitrate with other variables

	Coefficients	*Standard error*	*t Stat*	*P-value*
Intercept	-0.265406511	0.999745	-0.265474207	0.793115971
Organic matter	0.062709125	0.050657009	1.237916048	0.228795915
Dissolved oxygen	0.66873053	0.669092118	0.999459584	0.328439028
Total Bacteria	6.52038E-09	8.73692E-09	0.746301029	0.463386552
Depth	-0.20089554	0.146607591	-1.370294252	0.184417029

Based on the previous table, the equation is the following:

y = -0.02 + 0.001 organic matter -0.006 DO -1.64E-10 bacteria + 0.01 depth

Organic matter coefficient and depth were positively correlated with H_2S, while the coefficient of DO and bacteria are negatively correlated with H_2S (Table 14). Interaction between variables based on the regression equation shows that increasing organic matter and depth and decreasing DO and bacteria will increase H_2S concentration.

Table 14

Multiple linear regression analysis between H_2S with other variables

	Coefficients	*Standard error*	*t Stat*	*P-value*
Intercept	-0.022549555	0.03727786	-0.604904781	0.551431152
Organic matter	0.001766413	0.001888867	0.93517065	0.359852609
Dissolved oxygen	-0.00646997	0.024948684	-0.259331112	0.79779102
Total bacteria	-1.64542E-10	3.25777E-10	-0.505075748	0.61852799
Depth	0.012485791	0.005466611	2.284009349	0.032383742

Based on the table, the equation can be made as follows:

y = -0.02 + 0.001 organic matter -0.006 DO -1.64E-10 bacteria + 0.01 depth

Organic matter coefficient and depth positively correlated with H_2S, while the coefficient of DO and bacteria were negatively correlated with H_2S. The interaction between variables based on the regression equation showed that increasing organic matter and depth and decreasing DO and bacteria will increase H_2S concentration.

Organic matter. The highest organic matter content at the study site is at a depth of 0-20 m. According to Saru et al (2017), the mangrove area directly bordering the sea will experience a decrease in the content of sedimentary organic matter due to waves that dismantle sedimentary material directly into the sea, carried by the current. Organic material at stations I and III presented no significant differences, while at station II, organic material had higher levels. That is because Station II was in the middle of the mangrove area, being rich in litter and well suspended. Dewi et al (2018) reinforce the belief that the falling litter has a small amount of nutrients because it has not been decomposed.

Organic materials between depths have different levels, where with increasing depth, organic matter level is lower. According to Saibi & Tolangara (2017), the depth of the soil determines the levels of organic matter, with the highest levels of organic matter are in the upper soil layer, the first 20 cm (15-20%), decreasing in lower layers. According to Nugroho et al (2013), a depth of 20 cm is a strategic place for organisms to multiply and make photosynthesis. The type of substrate, mangrove density and abundance of organisms affect the content of organic matter in mangrove areas. Physical, chemical and biological factors interact in the degradation process. In the mangrove area of Bedono Village, the temperature is suitable for mangrove growth, the optimum temperature range being 26-30°C (Farhan & Razif 2017). Generally, the maximum decomposition process occurs at temperatures between 30 and 35, up to 45°C. Measured DO is below the average quality standard for mangroves. Ronavia et al (2020) state optimum DO level for mangroves is 4.1 mg L^{-1} - 6.6 mg L^{-1}, while the minimum level still within the tolerance limit is 4 mg L^{-1}. The Decree of the State Minister for Environment Number 51 of 2004 states that the optimum DO value for marine biota must be more than 5 mg L^{-1}. According to a statement of Schaduw (2018), mangroves can grow and develop well in a pH range between 6.2 and 8, while the optimum salinity level of *R. mucronata* stands is between 32-36‰ (Kaliu 2018).

Organic material in the Mangrove Bedono area is classified in a high category, ranging from 16.39 to 24.48%. Content of organic matter <3.5% in the sediment is considered very low, between 3.5 and 7% is relatively low, and 7-17% is moderate, 17-35% is high and >35% is very high (Indrawati et al 2013). In the decomposition process, there will be a release of carbon dioxide, where the higher the activity of microorganisms it can accelerate the process of decomposition of organic matter. According to Ostrownska & Grazyna (2014), the existence of N input will increase the primary production and storage of C soil. The higher the N-total content formed will cause a decrease in the C/N so that the mineralization process occurs.

Nitrate. Nitrate in the Bedono village mangrove area varies at each depth in each zoning. The highest nitrate content is at a depth of 0-20 cm at station II, and the lowest at 40-60 cm at station III. There is no difference in nitrate levels between stations I and II, while at station III, nitrate has lower levels. That is because Station III is close to land and lacks nutrient-rich water. According to Putri et al (2019), the erosion process of land allows nitrates previously trapped in the ground to enter the river and empty into the sea. Stations I and II are close to river and sea waters, where the high levels of nitrates at the two stations are suspected to occur because of currents carrying organic matter containing nitrate. In addition, stations I sand II have a higher mangrove density than station III. According to Song et al (2015), the addition of nitrogen can increase the availability and activity of soil enzymes, thereby accelerating litter decomposition. This is reinforced by Satriani (2016), who notes that in litter undergoing decay, the total nitrogen content released will be absorbed by the mangrove, while the tide will carry some to the surrounding waters, and some will disappear in the form of N_2.

Nitrates between depths have different levels. Some conditions cause nitrate levels to vary. According to Jatiswari et al (2022), nitrate content in sediments generally tends to be small because nitrates are first used by phytoplankton and other organisms. This is because the nitrate will dissolve faster in water, so particles that settle in sediments tend to be small.

The results of the PCA analysis showed a high correlation between nitrates with depth, H_2S, organic matter, bacteria, and DO in the first component, which explains 45.34% of total variety. In the multiple linear regression analysis, the significance value is less than 0.05, showing that depth, organic matter, DO and bacteria affect the nitrate content. With increasing depth, the level of nitrate decreases. Nutrient concentrations that are soluble in waters are usually lower than the concentration of nutrients in sediment. This is due to the activity of aerobic bacteria that exist around the roots of mangroves (Silvia et al 2014). These bacteria can dissolve nutrients in complex forms into sediments. The nitrate content in sediment is also influenced by the nitrification process by bacteria.

Based on the nitrate content obtained, the location of the study is classified with low fertility. According to Supriyantini et al (2018), nitrate content <2.27 mg 100 g^{-1} classifies a low fertility zone, a content between 2.27-11.29 mg 100 g^{-1} classifies a medium fertility of the zone, while a content between 11.3-112.5 mg 100 g^{-1} shows a high fertility. According to Widiyanti et al (2018), there are several factors that affect the nitrate content in the sediment, including sediment type, sizes and resuspension speeds. Nitrate levels in the euphotic area are strongly influenced by nitrate transportation, ammonia oxidation by microorganisms and nitrite-oxidizing bacteria (Mellbye et al 2016).

H_2S. The content of H_2S in the Bedono village mangrove area is higher with increasing depth. In this research, H_2S has the highest level at a depth of 40-60 cm. The high content is allegedly due to an anaerobic process in the decomposition of sediment organic material that causes the formation of H_2S. According to Sa'diyah et al (2018), the decomposition of organic matter by bacteria requires adequate oxygen. If the supply of DO is not sufficient, then the decomposition process becomes anaerobic and produces H_2S. Park et al (2020) state that when the basic conditions turn anoxic, sulfate-reducing bacteria will produce H_2S.

H_2S between depths has different levels. It can be assumed that the concentration of H_2S on the surface of the sediment is still experiencing circulation and diffusion well. Thus, DO demand was fulfilled on the surface layer for the aerobic process. According to Sakai et al (2013), the occurrence of physical mixing, driven by tides and the entry of seawater, produces a sufficient supply of DO, whereas DO is low in the sedimentary layer underneath, because circulation runs slowly. According to Asaoka et al (2018), in anoxic conditions, H_2S is produced through sulfate reduction in waters by sulfate-reducing bacteria using sedimentary organic material as reducing agents and energy sources.

There was a high correlation between H_2S and depth, nitrate, organic matter, bacteria and DO in the first component, which explains 45.34% of the total variety. In the multiple linear regression analysis, the significance value is less than 0.05, showing that depth, organic material, DO, and bacterial factors affect the H_2S content. Generally, H_2S is produced from the decomposition of organic matter. According to Waldah (2021), H_2S, which decomposes partially, remains in the soil and is partly released into the air as a gas. H_2S in the sediment will be assimilated into bacterial cells and oxidized to obtain energy. The oxidation results in sulfide compounds in the form of sulfate ions. The ions will be released by cells and utilized by plants (Bahera et al 2014).

H_2S concentration at the study site, according to the class III water quality standards, has exceeded the quality standard, because it is higher than 0.002 mg L^{-1}. H_2S is toxic to almost all types of aquatic organisms in the US-EPA criteria, if the H_2S concentration exceeds 0.002 mg L^{-1} (Acha et al 2018). The LC50 value for H_2S ranges from 0.002 to 6 mg L^{-1} for most sedimentary invertebrates (Kinsman-Costello et al 2015). The high concentration of H_2S indicates that the degradation of organic material is anaerobic and it is also a result of reduction of sulfate in anaerobic conditions by microorganisms (Jung et al 2020). The formation of H_2S can cause DO in the waters to be low and oxygen demand in the sediment to increase, producing toxins in the mangroves. According to Parveen et al (2017), anoxic H_2S production causes strong toxicity in mangroves and reduces growth by disrupting the absorption of nutrients, photosynthesis and metabolism.

Total bacteria. The total bacteria in the Bedono village mangrove sediment had the highest value at a depth of 0-20 cm, 178×10^5 CFU mL^{-1}. The high abundance of bacteria occurs because the sampling area was in the middle of the mangroves, rich in the litter that falls and is well suspended. According to Hanifah et al (2020), calm waves and currents can allow bacteria to settle along with sediment particles.

There was a significant difference in the total bacteria among the stations and the depths. The high abundance of bacteria on the surface of the sediment is inseparable from the high organic matter. The lack of organic matter as an energy source can cause heterotrophic bacteria death. According to Tian et al (2018), the content of organic matter is directly proportional to the abundance of heterotrophic bacteria, because the population of heterotrophic bacteria mostly requires nutritional supply from organic matter as an energy source for cell growth. Decomposition is also determined by the type of organic matter or by the decomposer factor. C/N ratio is a measure of the process of decomposition of organic material, while bacteria are a component that carries out its decomposition activities (Kamaluddin et al 2022).

Heterotrophic bacteria that are on the surface are the determinants of the microbial organic material cycle. The accumulation of organic matter in the upper layer of sediment affects the total bacteria in the sediment. Saibi & Tolangara (2017) note that the levels of organic matter are influenced by the depth of the soil layer. The upper layer of 20 cm contains 15-20% organic matter, the content of organic matter decreasing with higher depth. According to Suriani et al (2013), one of the important factors in bacterial growth is the pH value. Bacteria require an optimum pH of 6.5-7.5 to grow optimally. The minimum and maximum pH values for the growth of most bacterial species are 4 and 9. The effect of pH on the growth of bacteria is related to enzyme activity. If the acidity of the soil is excessive, it will cause the soil to be very sensitive to biological processes. The results of research conducted by Rani et al (2018) show that heterotrophic bacteria can live during different physical conditions by showing significant variations according to physical and chemical parameters in the environment.

Mangrove density. In the mangrove area in Bedono Demak Village, 2 species of mangroves were identified, namely *A. marina* and *R. mucronata*. At Station II, *A. marina* has a high density, of 5867 ind ha^{-1}. The high density can affect the fall of the litter, which will decompose. This process produces organic matter and affects the organic carbon content in mangrove sediments. According to Tongkaemkaew et al (2018), litter falling will become part of the sediment. The litter is broken down by microorganisms and finally transformed into organic carbon compounds that help improve soil structure and provide nutrition for plants. Jennerjahn (2020) stated that the highest carbon savings in the mangrove ecosystem were found in the sediment. Mangrove sediment has the ability to store carbon higher than the mangrove tree itself.

Conclusions. The highest organic matter content was found in the 0-20 cm layer, classifying it in the high category. There are differences in the organic matter content between depths, possibly influenced by litter and decomposition processes. The highest sediment nitrate content was in the 0-20 cm layer, classifying it in the low fertility category. There are differences in nitrate content allegedly influenced by currents and decomposition processes. The highest sediment H_2S content was found at a depth 40-60 cm and has exceeded the quality standards. There is a difference in H_2S between depths suspected to occur because of anaerobic degradation of organic matter. The results of PCA and multiple linear regression analysis show that depth, organic matter, DO, and bacteria affect the content of nitrate and H_2S. The highest total bacteria and DO are in the 0-20 cm layer. The total bacteria and DO levels are getting lower with the increase in depth. This is allegedly due to the process of decomposition of organic matter that utilizes the role of bacteria and consumption.

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