Internasional Journal of Integrative Sciences (IJIS)
Vol.4, No.7, 2025: 1549-1562

Abundance of Bacterial Colonies in Aquatic Ponds in Purwodadi Botanical Garden Pasuruan

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ARTICLEINFO

Keywords: Abiotic, Aquatic, Bacteria, Botanical Gardens

Received : 21 May
Revised : 23 June
Accepted: 23 July

ABSTRACT

The Purwodadi Botanical Garden plays a significant role in conserving Indonesia's plant biodiversity, particularly dry lowland species. Among its collections are aquatic plants distributed across several ponds with varying abiotic conditions, such as physical and chemical parameters, which influence plant distribution. Additionally, biotic factors like bacteria serve as environmental bioindicators and contribute to the decomposition of organic matter. This study aimed to assess the abundance and morphological characteristics of bacterial colonies, and to examine the relationship between environmental parameters and bacterial abundance in selected ponds at the Purwodadi Botanical Garden. Observations and sampling were conducted on ten ponds, and bacterial growth was analyzed using the pour plate method. Colony density was expressed in colony-forming units per milliliter (CFU/mL). Results showed that bacterial colony densities ranged from 35 to 103 CFU/mL, with colonies exhibiting round or rhizoid shapes, white coloration, and smooth or wavy edges. Bacterial abundance was positively influenced by higher light intensity and near-neutral pH levels. These findings contribute to a better understanding of microbial-environment interactions and provide useful insights for the ecological management of aquatic plant habitats in the Purwodadi Botanical Garden

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DOI: https://doi.org/10.55927/ijis.v4i7.409
E-ISSN: 2985-9050
https://mryformosapublisher.org/index.php/ijis/index

INTRODUCTION

Botanical gardens are key institutions for ex-situ plant conservation in Indonesia, playing a crucial role in preventing plant extinction (Sari et al., 2004). One prominent example in East Java is the Purwodadi Botanical Garden (Kebun Raya Purwodadi, KRP), located in Purwodadi Village, Purwodadi District, Pasuruan Regency. It is situated along the main road connecting Surabaya and Malang at kilometer 65. KRP lies at the foot of Mount Baung at an elevation of 300 meters above sea level, with geographic coordinates of 7°47'54.96"S and 112°44'18.28"E (Soegiarto, 2001). Beyond its conservation role, the botanical garden also serves educational purposes by showcasing plant diversity and enhancing public awareness and knowledge of Indonesia's flora (Sari et al., 2004). A botanical garden is typically characterized by a documented collection of living plants, supported by seed banks and herbarium specimens as complementary resources (Irawanto, 2011).

The Purwodadi Botanical Garden covers an area of 845,148 m^2 and was originally divided into 25 plots (vaks) and two main garden zones, separated by a central road. Each zone was further divided into three sections or sub-areas (Soegiarto, 2001). According to Laksono (2008), as the garden developed, it was reorganized into two major areas comprising six sub-zones, and the number of vaks increased from 25 to 183. Typically, a single vak contains plant species from multiple families or tribes; however, it is also possible for one plant family to occupy several vaks, depending on the number of specimens or individuals within that family. In addition to its terrestrial collections, KRP also maintains a diverse assemblage of aquatic plants distributed across 32 ponds. Each pond is characterized by distinct abiotic conditions—namely, variations in physical and chemical parameters—which influence the composition of aquatic plant species inhabiting them.

Abiotic factors refer to environmental conditions that influence aquatic life, including temperature, conductivity, water current, turbidity, light intensity, pH (acidity), dissolved oxygen, salinity, and total organic matter. These parameters also affect the presence and activity of microorganisms, which serve as key biological indicators in pond water ecosystems. Variations in the physical and chemical properties of each pond contribute to the diversity of bacterial communities inhabiting them (Mudatsir, 2007).

LITERATURE REVIEW

As organisms with widespread habitats, bacteria present in pond water also function as environmental bioindicators. In addition to the remediation potential of aquatic plants, bacteria are believed to contribute to the detoxification of polluted environments. Given that the ponds in the Purwodadi Botanical Garden are largely interconnected with plant life, bacteria are also thought to play a significant role in the natural decomposition of organic matter. Considering the diverse biological activities occurring within these aquatic environments, it is important to assess the abundance of microorganisms as a preliminary step in understanding their role in addressing environmental issues.

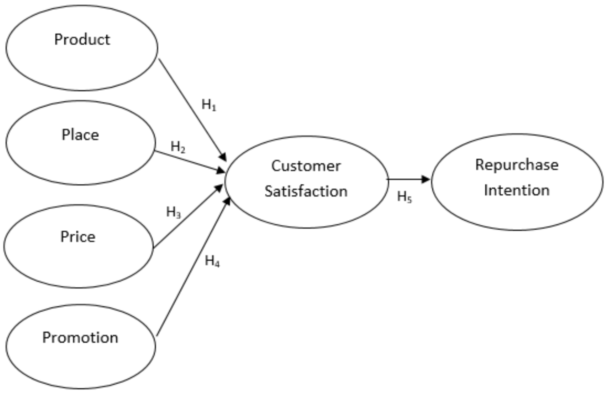

Figure 1. Conceptual Framework (The image has to be in good quality)

METHODOLOGY

This study employed an exploratory, descriptive, and quantitative approach to assess the abundance and morphological characteristics of bacterial colonies in water samples collected from ponds at the Purwodadi Botanical Garden. Samples were obtained from ten ponds located in vak II.C (pond 1), vak III.B (ponds 2 and 3), and vak XII.G (ponds 4 through 10). Data collection was conducted between July 4 and August 4, 2022, followed by laboratory analysis.

The equipment used in this study included a dipper for collecting pond water samples, sterile glass bottles for sample storage, test tubes and racks for serial dilution, and petri dishes for bacterial isolation. Additional tools included 100 mL measuring cylinders for preparing distilled water, 250 mL reagent tubes for media preparation, micropipettes with sterile tips for transferring sample suspensions, an autoclave for wet sterilization, and a dry oven for dry sterilization. Analytical scales were used to weigh media components, while 70% alcohol and tissue were used to sterilize the workspace. Spatulas were used to handle dry media, and a Bunsen burner with a lighter was employed for aseptic techniques. A hot plate with a magnetic stirrer was used to aid in dissolving the media. The materials used in this research included water samples from ponds 1 to 10 at the Purwodadi Botanical Garden, Plate Count Agar (PCA) medium, distilled water (aquades), and laboratory-grade Parafilm.

Water sampling was conducted using a random sampling method. Each sample was collected in a 200 mL measuring container, and environmental parameters such as temperature, pH, and light intensity were measured on-site. The collected water samples were then subjected to serial dilution and cultured using the pour plate technique. Bacterial colony counts were determined using the Total Plate Count (TPC) method. The resulting data were analyzed to evaluate the relationship between environmental conditions and both the abundance and morphological characteristics of bacterial colonies. According to Saputri et al. (2016), bacterial abundance can be calculated using the Total Plate Count (TPC) method with the following formula:

$$N\ (\mathrm{CFU/mL}) = \frac{\sum n \times 10^{x} \times 1000}{\text{volume inokulasi}}$$

Description:

N = bacterial abundance (CFU/mL)

n = number of bacterial colonies on the agar plate

10x = dilution factor

RESULTS AND DISCUSSION

Describe your research findings according to the research problem and purpose of the study. Discuss your findings according to the perspective of theory, concept or previous findings. Should describe this section in a comprehensive, simple and detailed manner. The author can make subchapters in this section.

The Purwodadi Botanical Garden serves as a center for biodiversity conservation and research, featuring 32 ponds distributed throughout the garden area (Irawanto, 2016). These freshwater ponds are supplied with flowing river water, providing a suitable habitat for various aquatic organisms, particularly aquatic plants (Hidayatullah et al., 2018). Aquatic plant collections are found in ten of these ponds, each hosting different plant species, as presented in Table 1.

Table 1. Locations of Ponds and Associated Aquatic Plant Species in the Purwodadi Botanical Garden

No.	**Pond**	**Location/Vak**	**Aquatic Plant Species Present**
1.	Pool 1	II.C	*Nymphaea rubra, Cyprinus sp.*
2.	Pool 2	III.B	*Lasia spinosa, Cyperus alternifolius, Echinodorus radicans, Actinoscirpus grossus, Thalia geniculata, Typhonodorum lindleyanum, Ludwigia octovalvis, Colocasia esculenta.*
3.	Pool 3	III.B	*Nymphaea rubra, Cyprinus sp.*
4.	Pool 4	XII.G	*Nelumbo nucifera, Typhonodorum lindleyanum, Nymphaea rubra, Cyprinus sp.*
5.	Pool 5	XII.G	*Lemna minor, Cyprinus sp.*
6.	Pool 6	XII.G	*Lemna minor.*
7.	Pool 7	XII.G	*Lemna minor, Pistia stratiotes.*
8.	Pool 8	XII.G	*Thalia geniculata, Lemna minor, Pistia stratiotes.*
9.	Pool 9	XII.G	*Ceratophyllum demersum.*
10.	Pool 10	XII.G	*Lemna minor, Pistia stratiotes, Nelumbo nucifera.*

Bacterial colonies formed on the agar plates were counted within the range of 30 to 300 colonies, in accordance with the criteria outlined by Safrida et al. (2021). The Total Plate Count (TPC) method involves diluting the sample with sterile saline solution until the bacterial concentration allows for accurate enumeration. Colony counts below 30 are considered statistically unreliable, as they may not adequately represent the sample. Conversely, counts exceeding 300 colonies are typically excluded, as the colonies tend to overlap, making it difficult to distinguish individual colony-forming units.

Table 2. Total Bacterial Counts (CFU/mL) in Ponds at the Purwodadi Botanical Garden (KRP)

Pond	Repeat	Colony Count	Total Bacteria (CFU/mL)	Average (CFU/mL)
1	1	18	TSUD	6,0 × 10^4
	2	60	6,0 × 10^4	
2	1	8	TSUD	7,9 × 10^4
	2	79	7,9 × 10^4	
3	1	19	TSUD	7,7 × 10^4
	2	77	7,7 × 10^4	
4	1	89	9,0 × 10^4	9,5 × 10^4
	2	100	1,1 × 10^5	
5	1	35	3,5 × 10^4	6,9 × 10^4
	2	103	1,0 × 10^5	
6	1	41	4,1 × 10^4	6,8 × 10^4
	2	94	9,4 × 10^4	
7	1	90	9,0 × 10^4	7,6 × 10^4
	2	61	6,1 × 10^4	
8	1	90	8,9 × 10^4	9,8 × 10^4
	2	106	1,1 × 10^5	
9	1	54	5,4 × 10^4	7,4 × 10^4
	2	93	9,3 × 10^4	
10	1	18	TSUD	7,7 × 10^4
	2	77	7,7 × 10^4	

The total abundance of bacterial colonies was calculated by multiplying the number of colonies formed on the agar plate by the dilution factor and dividing the result by the inoculated volume. The final value is expressed in Colony Forming Units per milliliter (CFU/mL). Based on the data presented in Table 2, the highest average bacterial count was recorded in water samples from pond 8, with a total of 9.8 × 10^4 CFU/mL. In contrast, the lowest average count was observed in pond 1, with a total of 6.0 × 10^4 CFU/mL.

Table 3. Morphological Characterization of Bacterial Colonies from KRP Ponds on Plate Count Agar (PCA) Medium

Pond	**Colony Shape**	**Color**	**Edge Type**	**Elevation**
Pond 1	Round	White	Smooth	Convect
Pond 2	Round	White	Smooth	Flat
Pond 3	Round	White	Smooth	Flat
Pond 4	Round	White	Smooth	Flat
Pond 5	Round	White	Smooth	Convect & Flat
Pond 6	Round	White	Smooth	Flat
Pond 7	Rhizoid	White	Smooth	Flat
Pond 8	Rhizoid	White	Smooth	Flat
Pond 9	Rhizoid & Round	White	Smooth & Wavy	Convect & Flat
Pond 10	Round	White	Smooth	Convect

The morphological characteristics of bacterial colonies observed in this study include shape, color, edge type, and elevation. Two distinct colony shapes were identified: round and rhizoid. Rhizoid-shaped colonies were observed in samples from ponds 7, 8, and 9. Most of the cultivated bacterial colonies exhibited smooth edges, although fibrous (filamentous) edges were also observed, particularly in colonies from pond 5. With respect to elevation, two types were recorded: convex and flat. All bacterial colonies exhibited white pigmentation across all samples. Based on the data presented in Table 3, round-shaped colonies were the most dominant, while rhizoid shapes were found exclusively in ponds 7, 8, and 9. The morphological diversity of the colonies observed from the cultivation process is illustrated in Figure 1.

The bacteria that grew in this study were not taxonomically specific, as Plate Count Agar (PCA) is a general-purpose medium that supports the growth of a wide range of microbial species. As noted by Hiaranya (2017), PCA is suitable for total microbial enumeration due to its nutrient-rich composition. It contains casein enzymic hydrolysate, which supplies amino acids and other complex nitrogenous compounds, as well as yeast extract, which provides B-complex vitamins. These components make PCA an effective medium for cultivating various types of bacteria. Bacteria grown on PCA are typically generalists and exhibit common morphological characteristics, including white coloration, round colony shapes, smooth edges, and either flat or convex elevations.

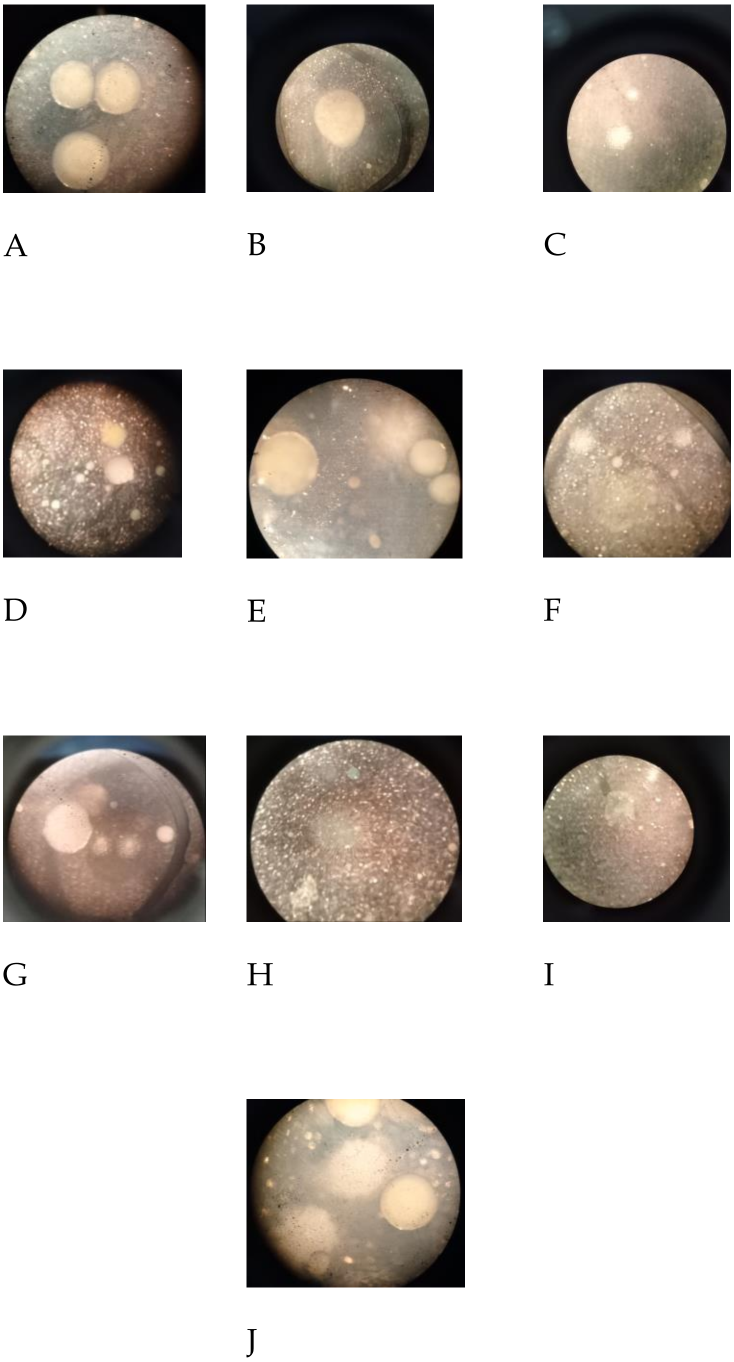

Figure 1. Colony morphology of isolated bacteria from pond water samples: (A) Pond 1, (B) Pond 2, (C) Pond 3, (D) Pond 4, (E) Pond 5, (F) Pond 6, (G) Pond 7, (H) Pond 8, (I) Pond 9, (J) Pond 10. Images captured at 1000× magnification (Personal documentation, 2022).

Data collection was conducted on 10 selected ponds out of the total 32 ponds located within the Purwodadi Botanical Garden. Not all ponds are currently active, but those situated in visitor-accessible zones—such as Neighborhoods I, II, III, and XII—remain functional. These active ponds serve as habitats for 13 aquatic plant species maintained by the botanical garden. Water entering the ponds originates from residential runoff in the surrounding areas of the Purwodadi Botanical Garden. This inflow often carries household waste and, in some cases, potential industrial pollutants, as the garden is located near an industrial zone. Human activity in the selected neighborhoods is generally more intensive than in other parts of the garden. The range of anthropogenic activities observed around ponds 1 to 10 was a key factor in selecting these sites for data collection. According to Pratiwi and Rachmadiarti (2021), human activities in aquatic environments frequently contribute to environmental degradation, particularly through the direct disposal of untreated waste into waterways.

The water quality parameters measured in each pond included water temperature and pH, while the environmental parameter observed around each pond was light intensity. The average water temperature across all ponds was 30.8°C, with the highest temperature recorded in pond 8 at 34.9°C. In contrast, pond 1 exhibited the lowest water temperature, which corresponded with the lowest light intensity recorded in its surrounding environment, at 2,060 cd. For the acidity parameter (pH), the highest pH value of 8.3 was observed in ponds 3 and 9. Meanwhile, the lowest pH was recorded in pond 5, with a value of 6.0.

The variation in environmental conditions surrounding the ten ponds at the Purwodadi Botanical Garden affects the values of measured environmental parameters. These ponds are colonized by various species of aquatic plants, and some are partially shaded by surrounding vegetation. Pond 1 exhibited the lowest temperature and light intensity among all ponds, primarily due to the shade provided by a Ficus tree directly above the pond. Additionally, the pond is densely vegetated with *Nymphaea* species, further contributing to the cooler microclimate in its surroundings. In contrast, the remaining ponds are not directly shaded by trees, although they are also populated by diverse aquatic plant species.

The metabolic activity of organisms inhabiting the pond environment—particularly fish—contributes significantly to variations in environmental parameter values. According to Wahyuningsih and Gitarama (2020), both fish feed and their metabolic waste serve as major sources of nitrogen in aquatic environments. Approximately 70% of the feed consumed by fish is excreted in the form of ammonia and organic proteins as end products of metabolism. Of the total nitrogen excreted, around 90% is in the form of ammonia, while the remaining 10% is excreted as urea. Ammonia present in the water is measured as total ammonia, which includes both unionized ammonia (NH_3) and ionized ammonium (NH_4^+). These compounds influence key water parameters such as pH and temperature. Free ammonia (NH_3), which exists in an un-ionized form, is known to be toxic to aquatic organisms.

Free ammonia (NH_3) is non-ionized and exhibits toxicity to aquatic organisms, whereas ammonium (NH_4^+) exists in ionized form and is generally less harmful. Elevated concentrations of ammonia in aquatic environments may not only result from the metabolic activity of aquatic fauna, such as fish, but also serve as an indicator of organic pollution originating from domestic sewage, industrial effluents, and agricultural fertilizer runoff (Firdaus et al., 2018). Due to the diverse sources of ammonia input, changes in pH can occur through biochemical processes. As noted by Marsono (1996, in Cahyani, 2021), the oxidation of ammonia into nitrate via nitrification releases hydrogen ions (H^+), which contributes to a decrease in pH levels. Therefore, variations in acidity among different pond environments can be attributed to the intensity of nitrification activity mediated by aquatic microorganisms.

The pond water in the Purwodadi Botanical Garden is sourced from external drainage channels, which are presumed to originate from residential and industrial catchment areas. It is suspected that domestic and industrial effluents have contributed to the contamination of river water that ultimately flows into the Botanical Garden. This contamination is believed to influence the fluctuations in pond water pH. Additionally, variations in pH can also be attributed to changes in carbon dioxide concentrations within the water body. Aquatic ecosystems undergo dynamic carbonate equilibrium processes, driven by the photosynthetic and respiratory activities of resident organisms. During respiration, elevated CO_2 production shifts the carbonate equilibrium toward increased hydrogen ion (H^+) release, resulting in a decline in pH. Conversely, during photosynthesis, CO_2 is actively consumed, driving the reaction in the opposite direction and leading to a rise in pH levels.

In the study by Komala et al. (2012), pH levels significantly influence bacterial growth, with most bacterial species thriving within a pH range of 5.3 to 7.5. Nevertheless, many microorganisms possess adaptive mechanisms that enable survival under both acidic and alkaline conditions. In the ponds of the Purwodadi Botanical Garden, pH measurements ranged from 6.0 to 8.3, with an average value of 7.4. These values fall within a range considered suitable for supporting microbial life. In addition to pH, ambient temperature also plays a crucial role in regulating bacterial growth and activity. Furthermore, fluctuations in pH may result from bacterial metabolic processes themselves. The pond environments in the Botanical Garden are shaded by various tree species, and the decomposition of fallen leaves contributes to organic matter input, which is subsequently broken down by microbial activity. This decomposition process can influence the chemical composition of the water, including its acidity. The relationship between pH and bacterial growth is illustrated in Figure 2.

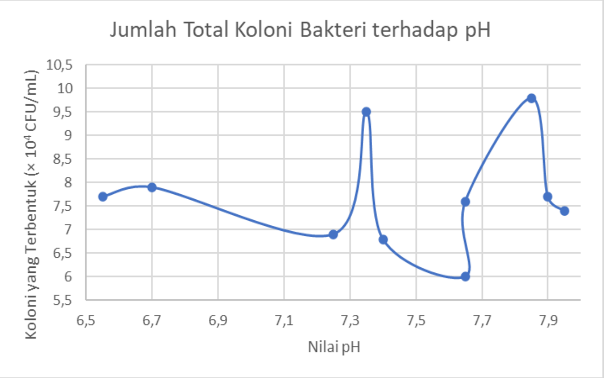

Figure 2. Graph illustrating the effect of pH on bacterial growth based on field data (Source: Personal Data)

Figure 2 illustrates that bacterial growth becomes more optimal as the pH approaches neutrality. Suradi (2012) emphasized that microbial proliferation tends to progress toward an optimal pH, with neutral conditions being the most favorable for the majority of bacterial species. The ideal pH range for bacterial growth is typically between 6.6 and 7.5, within which enzymatic activity and cellular metabolism operate most efficiently.

In the study by Mulyono (2014), the decomposition of organic matter in natural environments is accompanied by changes in pH, driven by microbial activity. In the initial stages of decomposition, the breakdown of organic compounds into organic acids—such as lactic acid—lowers the environmental pH, resulting in more acidic conditions. As the decomposition process progresses, the accumulation of acids diminishes, and the pH gradually returns to neutral. Hastuti (2011) further explains that pH is a key environmental factor influencing bacterial growth and activity, particularly for bacteria involved in ammonia oxidation. Autotrophic ammonia-oxidizing bacteria exhibit optimal growth at pH levels between 7.5 and 8.5. In contrast, heterotrophic bacteria tend to be more tolerant of acidic conditions and demonstrate higher growth rates and yields under environments with lower dissolved oxygen concentrations.

Based on their temperature preferences for optimal growth, bacteria are generally classified into two major groups: mesophilic and thermophilic bacteria. Mesophilic bacteria grow best within a temperature range of 25–30°C, with optimal enzymatic activity typically occurring around 40°C (Puspitasari et al., 2012). In contrast, thermophilic bacteria are adapted to higher temperatures, exhibiting optimal growth in the range of 45–80°C (Mahmudah et al., 2016). Temperature measurements of pond water in the Purwodadi Botanical Garden fall within the range of 26.3 to 36.2°C. Based on this environmental parameter, the bacterial community present in these ponds is likely dominated by mesophilic species. The relationship between temperature and bacterial growth is illustrated in Figure 3.

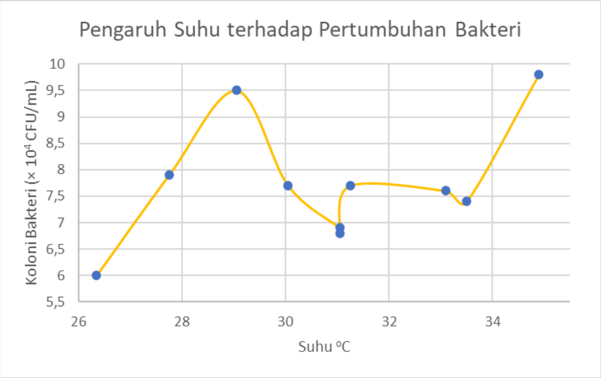

Figure 3. Graph illustrating the effect of temperature on bacterial growth based on field observations (Source: Personal Data)

High light intensity is generally correlated with increased ambient temperature. This observation aligns with the findings of Firdaus et al. (2018), who reported that elevated ambient temperatures are primarily driven by high solar radiation intensity. The relationship between light intensity and environmental temperature, as observed from environmental parameter measurements in the Purwodadi Botanical Garden, is illustrated in Figure 4.

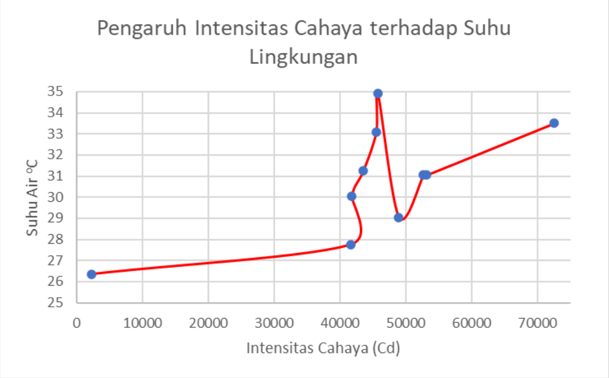

Figure 4. Graph illustrating the effect of light intensity on environmental temperature (Source: Personal Data)

The observational data presented in Figure 4 reveal a discrepancy with the statement by Firdaus et al. (2018), which suggested that higher light intensity is associated with increased ambient temperature. In the case of Pond 9, despite recording higher light intensity values, the water temperature was observed to be lower compared to Pond 8, which exhibited lower light intensity but higher temperature. This anomaly may be attributed to differences in biological activity and vegetation composition between the two ponds. Pond 8 is predominantly covered by Lemna minor, with a few individuals of Pistia stratiotes and Thalia geniculata located in one corner. In contrast, Pond 9 is extensively colonized by Ceratophyllum demersum, a submerged aquatic plant species. According to Puspitaningrum et al. (2012), Ceratophyllum demersum has a higher capacity for oxygen production compared to Lemna minor, contributing to greater levels of dissolved oxygen in the water. Since rising water temperatures are known to reduce the solubility of oxygen, the elevated oxygen saturation in Pond 9 may be associated with lower water temperatures, suggesting that higher dissolved oxygen levels may have a cooling effect on the aquatic environment. This finding underscores the complex interplay between light intensity, aquatic vegetation, and temperature regulation in freshwater ecosystems.

The study data indicate that temperature plays a significant role in influencing the total number of bacterial colonies. In particular, samples from Pond 8, which had an average water temperature of 34.9°C, exhibited a total bacterial count of 9.5×10^4 CFU/ml. According to Firdaus et al. (2018), elevated temperatures can enhance microbial metabolic activity, thereby accelerating the decomposition of organic matter. This process provides additional substrates for microbial growth, contributing to the observed increase in bacterial colony numbers under higher temperature conditions.

Microscopic observation of bacterial colony morphology revealed that the majority of colonies exhibited round shapes with white pigmentation and a range of edge characteristics, including smooth and glistening margins. Based on the analysis of environmental conditions and presumed biological activity in the pond ecosystems, the isolated bacteria are suspected to belong to groups commonly associated with environmental indicator species. Several genera of bacteria known to function as environmental indicators and capable of thriving in mesophilic conditions include Bacillus, Listeria, Pseudomonas, Vibrio, Nitrobacter, and Enterobacter. According to Ashari (2021), Pseudomonas spp. play an important role in the decomposition of organic compounds, particularly in the nitrification process, wherein ammonia is oxidized into nitrites and subsequently into nitrates, facilitating its dissolution in aquatic environments such as seawater and sediments. Furthermore, based on the macroscopic observations reported by Nainggolan et al. (2015), colonies of the genus Nitrobacter are characterized by a milky white coloration and undulated edges. Other nitrifying bacteria such as Nitrospina and Nitrococcus typically display smooth, rounded colony surfaces, whereas Nitrospira colonies tend to have irregular surface morphologies.

CONCLUSION AND RECOMMENDATION

The total bacterial colony density in pond water samples from the Purwodadi Botanical Garden ranged from 35 to 103 CFU/mL, with the highest density recorded in samples from Pond 8. Macroscopic morphological characteristics of the bacterial colonies, as observed under a light microscope, included round and rhizoid shapes, white pigmentation, smooth to wavy colony edges, and elevations that were either flat or convex. The abundance of bacterial colonies was strongly influenced by environmental conditions. Higher light intensity at specific locations was associated with elevated ambient temperatures, which in turn correlated with increased bacterial abundance. Furthermore, pH levels also played a role, with bacterial abundance tending to be higher in environments where pH values approached neutral, which supports optimal growth conditions for a wide range of bacterial taxa.

FUTHER STUDY

Further research is recommended to enhance the identification of bacterial communities present in the pond ecosystem of the Purwodadi Botanical Garden using molecular approaches, such as 16S rRNA gene analysis, to achieve more accurate taxonomic classification. The results of such studies are expected to contribute significantly to advancing knowledge on the ecological roles of bacteria within pond ecosystems and to explore their potential as bioindicators for environmental monitoring and sustainable water quality management.

ACKNOWLEDGMENT

The author extends sincere gratitude to all individuals who contributed to the completion of this research. Special appreciation is given to colleagues at the Purwodadi Botanical Garden for their invaluable support throughout the study. Deepest thanks are also conveyed to previous researchers and internship students (Khumairoh Nur Abidah and Aprilya Tri Rahmawati) whose work has laid a critical foundation for this study.

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