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The Dual Role of Vetiver Root Extract on Heterotrophic Bacterial Growth in Lake Riparian Zones: Implications for Lake Riparian Zone Water Quality

Nina Hermayani Sadi[1*)], Ni Made Diah Puspita Dewi[2], I Wayan Suirta[2]

[1]Research Center for Limnology and Water Resources, National Research and Innovation Agency (BRIN) Republic of Indonesia, Cibinong, Indonesia
[2]Departement of Chemistry, Udayana University, Badung Regency, Bali, Indonesia

*) Corresponding author's e-mail: nina006@brin.go.id

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Abstract: Plant–microbe interactions are fundamental to maintaining ecological balance in aquatic systems. In riparian zones, fluctuating lake water levels create standing water conditions that facilitate exchanges between riparian vegetation and aquatic microbes, particularly heterotrophic bacteria. This study examined the influence of Vetiveria zizanioides (Vetiver) root extract on heterotrophic bacterial growth in riparian ecosystems. Vetiver, widely utilized for erosion control and sediment stabilization, was selected as the experimental species. Soil and water samples from two small lakes in Cibinong, Bogor, West Java (Situ Cibuntu and Situ Cibinong), served as sources of bacterial isolates. Vetiver root extract was prepared via aqueous extraction and applied at varying concentrations in 50% tryptone glucose yeast (TGY) liquid medium. Bacterial growth responses were assessed through optical density measurements, alongside environmental parameter evaluations. Results demonstrated that Vetiver root extract modulated bacterial growth in a concentration-dependent manner: a 25% extract concentration significantly promoted heterotrophic bacterial growth, suggesting a role in enhancing nutrient cycling, whereas higher concentrations exhibited inhibitory effects, implying potential applications for microbial population management. These findings highlight vetiver root extract's dual role in stimulating or suppressing microbial activity within riparian ecosystems. This research provides a scientific foundation for developing nature-based solutions (NBS) to promote lake ecosystem stability and environmental sustainability. Further research is needed to assess the long-term effects of Vetiver root extract application on soil health, water quality, plant-microbe interactions, and overall ecosystem sustainability.

Keywords: bacterial growth, heterotrophic bacteria, riparian zone, root extract, vetiver plants

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1. Introduction

Lakes are stagnant aquatic ecosystems occupying basins where inflow exceeds outflow, resulting in dynamic water levels that fluctuate over time (UNEP-IETC/ILEC, 2001). As lake water levels rise, the lakeside area (commonly referred to as the riparian zone) becomes inundated. This transitional area often hosts diverse plant species that serve as ecological buffers between terrestrial and aquatic ecosystems (Leatemia., et al, 2016). These conditions foster interactions between riparian plants and aquatic microorganisms, such as heterotrophic bacteria carried by the lake water.

Heterotrophic bacteria are microorganisms incapable of synthesizing their food through photosynthesis or chemosynthesis. Instead, they rely on organic material from the environment, including detritus from

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decomposing organisms or anthropogenic waste, as energy sources and carbon (Notowinarto and Agustina, 2015). Riparian plants, such as Vetiver grass (Vetiveria zizanioides), commonly referred to as Akar Wangi, contribute organic matter that can be metabolized by these microbes, enhancing nutrient cycling and improving soil structure. Vetiver grass (Vetiveria zizanioides), with its robust root system extending up to five meters, not only stabilizes soil but also enhances water quality by filtering sediments and nutrients (Jannah H and Safnowandi 2018). Additionally, the organic compounds released through its root exudates support microbial diversity and ecological processes such as nutrient cycling and decomposition of organic matter. Microbial interactions around plant roots also contribute to accelerating the decomposition of organic pollutant compounds, such as pesticides and hydrocarbons, into non-toxic forms (Bolan, 2011).

In the rhizosphere, plants and microbes interact by exchanging root exudates, which may serve as antimicrobial agents or carbon sources for microorganisms. Plants actively shape microbial communities by secreting specific exudate compounds, altering microbial composition and diversity to form beneficial associations (Broeckling, et al., 2008; Cesco, et al., 2012). These exudates' concentration and composition significantly influence the rhizosphere's microbial community structure (Cesco,et al., 2012). Their interactions with riparian plants often include symbiotic relationships where bacteria facilitate nutrient uptake and provide protection against soil-borne pathogens. Additionally, both plants and microbes engage in chemical signal exchanges, which affect gene expression and, consequently, the diversity and abundance of microbial and plant populations (Waters and Bassler, 2005; Zhuang, et al., 2013; Widyati, 2017). The diversity and abundance of microbial populations found around the roots of vetiver, as a result of ecological interactions, may include bacteria capable of detoxifying heavy metals. These microorganisms possess the ability to mitigate the harmful impacts of heavy metals through both intracellular and extracellular mechanisms within their tissues, potentially influencing the efficacy of bioremediation processes (Triyani & Hafsan, 2021).

This study examines the interaction between Vetiver root compounds and heterotrophic bacteria in riparian zones, particularly in response to fluctuating lake water levels. The aim is to assess the effects of Vetiver root extract on heterotrophic bacterial growth, which is evaluated by measuring cell density using the Optical Density (OD) method. This approach is used to test the hypothesis that biochemical compounds released by Vetiver roots influence bacterial communities. This research contributes to understanding plant-microbe interactions in riparian ecosystems and the ecological implications of root-derived compounds. This research provides a scientific foundation for developing nature-based solutions (NBS) to promote lake ecosystem stability and environmental sustainability. Study of Zhang, et al. (2021) and Arrijani, et al. (2024) found that selecting suitable plant species in riparian zones can enhance microbial communities, improve nutrient cycling, and support ecosystem resilience.

2. Materials and Method

2.1. Research Area

Situ Cibuntu and Situ Cibinong, two small lakes located in Cibinong, Bogor Regency, West Java, Indonesia, were selected as study sites. Situ Cibuntu, situated within the Cibinong National Research and Innovation Agency Complex (06°29'S-106°51'E), covers an area of 2.11 hectares with a maximum depth of 1.20 meters (Sulastri, et al., 2020; Sadi, 2013). Previous studies have indicated that the lake has experienced significant sedimentation due to high suspended solid inputs (Zulti, et al., 2012). Situ Cibinong, located near the Mayor Oking Highway and Cibinong's traditional market (06°28'50"S-106°49'36"E), originally covered 5.77 hectares but has since reduced to 4.5 hectares. The lake suffers from pollution caused by domestic and non-domestic waste (Askhary, 2016), despite being a popular fishing spot.

2.2. Water and Soil Sampling

Surface water samples were collected from three distinct points within the riparian zones of both Situ Cibuntu and Situ Cibinong. Sampling was conducted at three sites in each lake, selected based on variations in riparian conditions. Water samples were collected at a depth of 50 cm from the lake surface and stored in polyethylene bottles. For water quality analysis, 500 mL of each water sample was collected in polyethylene bottles and either refrigerated or acidified to pH 2 following standard protocols (APHA, 2005). Meanwhile, water samples for microbiological analysis were collected in sterile bottles. Water samples were refrigerated and processed for subsequent isolation within 24 hours, as recommended by Sadi (2013). Soil samples were collected using sterilized shovels and gloves. Samples were taken from areas adjacent to the water's edge and slightly drier locations to represent varying soil moisture conditions. The samples were stored in polypropylene plastic containers.

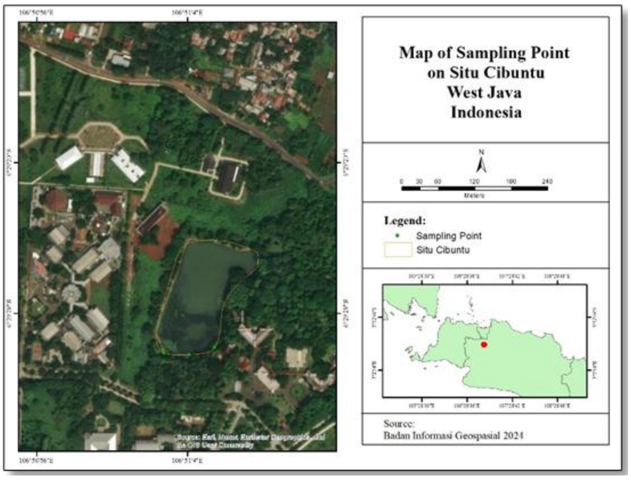

Figure 1. Sampling Location of Situ Cibuntu

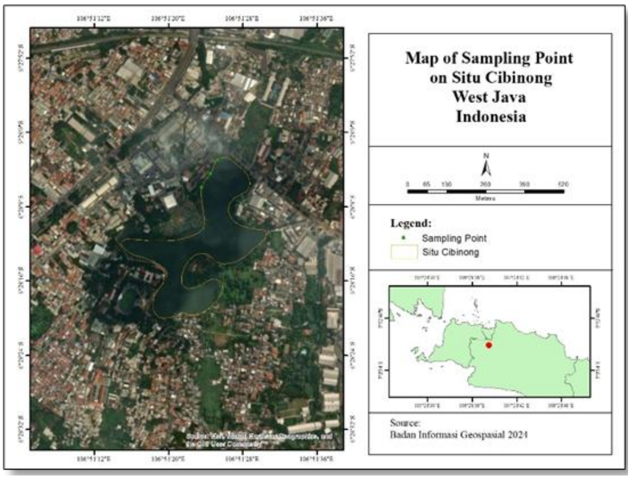

Figure 2. Sampling Location of Situ Cibinong

2.3. Vetiver Root Extraction

Vetiver root extract was prepared using a standard aqueous extraction method. Dried vetiver roots, sourced from Garut, were cleaned and oven-dried at 50°C for 24 hours. The roots were cut into 1 cm segments and added to an Erlenmeyer flask containing 750 mL of distilled water (a 1:15 solid-to-liquid ratio). The mixture was heated to 100°C for 30 minutes and filtered through a multi-step process involving coarse filter paper, 1.5 µm GF/C filter paper, and 0.7 µm GF/F filter paper to remove solid particles and colloidal impurities. The resulting filtrate with no particulate matter was considered the 100% vetiver root extract.

2.4. Effect of Vetiver Root Extract on Bacterial Growth

To investigate the impact of vetiver root extract on bacterial growth, 0.6 mL of water samples were inoculated into 6 mL of half-strength TGY medium supplemented with various concentrations of vetiver root extract (0%, 1.5625%, 3.125%, 6.25%, 12.5%, 25%, 50%, and 100%). The inoculated media were incubated at 150 rpm for 48 hours. Microbial growth was evaluated by the turbidimetric method, quantified using a UV-Vis spectrophotometer at 600 nm. Optical Density Units (ODU) were used to measure bacterial density. One-way ANOVA, followed by Tukey's HSD test, was employed to analyze the statistical significance of the results using PAST 4.03 software.

2.5. Heterotrophic Bacterial Enumeration

Water samples from the three sites were serially diluted at 10^{-2} and 10^{-4} dilutions. Aliquots of 100 µL of each diluted sample were spread plated onto TGY agar plates and incubated at room temperature for 48 hours. Colony-forming units (CFUs) were counted to determine bacterial abundance.

Soil samples (5 g) were added to 45 mL of peptone water and shaken for 1 hour at 150 rpm. Serial dilutions (10^{-4} and 10^{-6}) were prepared, and 100 µL aliquots were spread plated onto TGY agar plates. The plates were incubated at room temperature for 48 hours, and CFUs were counted.

2.6 Water and Soil Quality Analysis

Water quality parameters were measured using standard analytical methods, including pH, salinity, electrical conductivity, dissolved oxygen (DO), ammonium, total phosphate, total nitrogen, chemical oxygen demand (COD), nitrate, and nitrite. A portable water quality checker (EZ-9909) was used to determine pH, salinity, and electrical conductivity. DO levels were measured using a Lutron DO meter. Ammonium, total phosphate, total nitrogen, and COD were analyzed using spectrophotometric methods (phenate, persulfate-ascorbic acid, brucine, and closed reflux, respectively) (APHA, 2005). Soil pH was measured using an ATC pH meter. Soil organic carbon content was determined spectrophotometrically at a specific wavelength (APHA, 2005).

3. Result and Discussion

3.1. Environmental Condition in Situ Cibuntu and Situ Cibinong

Water Quality

Table 1 summarize water quality data for Situ Cibuntu and Situ Cibinong. The analysis revealed distinct differences in nutrient composition between the two locations. Situ Cibinong exhibited higher concentrations of ammonium, total nitrogen, and Chemical Oxygen Demand (COD), whereas Situ Cibuntu showed elevated nitrate, nitrite, and organic nitrogen levels.

Nitrogen is a crucial nutrient for plant growth, serving as a key component in protein synthesis and supporting aquatic plant development. In aquatic ecosystems, nitrogen compounds, such as nitrate (NO_3^-), ammonium (NH_4^+), and nitrite (NO_2^-), are naturally derived from the metabolic activities of aquatic organisms and the bacterial decomposition of organic matter (Boyd, 1979; Ibrahim, et al., 2021). These nitrogen forms significantly influence species diversity, abundance, and nutrient availability in aquatic ecosystems for flora and fauna (Horne & Goldman, 1994).

Inorganic nitrogen compounds, particularly nitrate (NO_3^-) and ammonium (NH_4^+), can enhance bacterial growth. Ammonium often originates from industrial or human organic waste entering water bodies, while nitrate is primarily produced through the metabolic processes of aquatic organisms. During these processes, ammonium is oxidized to nitrite, which is subsequently converted to nitrate by waterborne microorganisms (Amalia, et al., 2021). The highest ammonium concentration was recorded at Situ Cibinong (2.250 ± 0.82 mg/L), while the highest nitrate concentration was observed at Situ Cibuntu (0.980 ± 0.06 mg/L).

Table 1. Water Quality Data for Situ Cibuntu and Situ Cibinong

Sampling Location		NO_3-N Conc. (mg/L)	NH_4-N Conc. (mg/L)	NO_2-N Conc. (mg/L)	TN Conc. (mg/L)	N Organic Conc. (mg/L)	COD Conc. (mg/L)
Situ Cibuntu	1	0.915	0.849	0.247	2.665	0.654	61.833
	2	1.068	0.699	0.548	2.411	0.060	22.111
	3	0.966	0.537	0.378	3.057	1.176	18.000
	Average	**0.980**	**0.700**	**0.400**	**2.711**	**0.630**	**33.981**
	SD	**0.06**	**0.13**	**0.14**	**0.27**	**0.46**	**19.77**
Situ Cibinong	1	0.373	3.388	0.085	3.867	0.021	16.334
	2	0.484	1.513	0.065	3.369	1.307	49.834
	3	0.252	1.848	0.049	2.257	0.108	51.333
	Average	**0.370**	**2.250**	**0.007**	**3.160**	**0.480**	**39.167**
	SD	**0.09**	**0.82**	**0.01**	**0.67**	**0.59**	**16.16**

Conversely, nitrite (NO_2^-), part of the nitrogen cycle, can negatively impact microbial populations. Nitrite contamination from fertilizers, livestock waste, and other organic inputs can inhibit bacterial growth at concentrations exceeding 1 mg/L by interfering with bacterial respiration. Nitrite binds to cellular components like cytochromes, disrupting electron transfer during respiration (Radiastuti, 2009). Situ Cibuntu exhibited higher nitrite levels (0.400 ± 0.14 mg/L) compared to Situ Cibinong, potentially causing slight disturbances to microbial growth.

COD is another critical water quality parameter, representing the oxygen demand required by aerobic microbes to oxidize organic matter into biomass, carbon dioxide, water, and inorganic compounds. High COD values typically indicate elevated organic matter levels, leading to reduced dissolved oxygen availability (Rahmawati, et al., 2013). The COD concentration at Situ Cibinong (39.167 ± 16.16 mg/L) was significantly higher than at Situ Cibuntu, likely due to household wastewater and market waste contributing additional organic material to the water.

The data in Table 2 represent the measurements conducted during the sampling process. The observed differences in water temperature between Situ Cibinong and Situ Cibuntu reflect the environmental conditions at the sampling time. Water samples from Situ Cibuntu were collected earlier than those from Situ Cibinong. Additionally, the presence of dense vegetation and shaded areas surrounding Situ Cibuntu likely contributed to its lower water temperature than Situ Cibinong.

The pH values of the water samples from both Situ Cibuntu and Situ Cibinong were within the optimum range for microbial growth, thereby avoiding harmful effects on microorganisms. Specifically, the optimal pH range for bacterial growth is between 6.5 and 7.5, while the tolerable range extends from 4 to 9 (Hamdiyati, 2011). Furthermore, the measured values of Electrical Conductivity (EC) and salinity in both water bodies were relatively low, indicating minimal impact on bacterial growth.

Table 2. Water Quality Data from Situ Cibuntu and Situ Cibinong.

Sampling Location		**Temperature (˚C)**	**pH**	**Electrical Conductivity (µS/cm)**	**Total Dissolved Solid (ppm)**	**Salinity (%)**	**Dissolved Oxygen (ppm)**
Situ Cibuntu	1	26.40	6.81	144.7	72	0	4.40
	2	26.70	6.84	144.0	71	0	4.53
	3	26.70	6.80	144.0	72	0	4.60
	Average	**26.60**	**6.82**	**144.23**	**71.67**	**0.00**	**4.51**
	SD	**0.14**	**0.02**	**0.33**	**0.47**	**0.00**	**0.08**
Situ Cibinong	1	29.67	6.97	203.0	101	0.01	3.83
	2	29.90	7.21	156.0	78	0	5.60
	3	29.53	7.95	155.7	77	0	8.33
	Average	**29.70**	**7.38**	**171.57**	**85.33**	**0.00**	**5.92**
	SD	**0.15**	**0.42**	**22.23**	**11.09**	**0.00**	**1.85**

Dissolved oxygen (DO) is another critical factor for microbial survival, as aerobic microorganisms require oxygen to oxidize nutrients for metabolic processes. However, some microbial groups are anaerobic, with oxygen potentially toxic to their growth (Hamdiyati, 2011). The study results indicate that the dissolved oxygen concentrations in both Situ Cibuntu and Situ Cibinong were sufficient to support microbial activity and sustain their life processes.

Soil Condition of Situ Cibuntu and Situ Cibinong

The soil conditions of Situ Cibuntu and Situ Cibinong are summarized in Table 3. The organic carbon content in the soil sample from Situ Cibinong was higher than that observed in Situ Cibuntu. Organic carbon is a vital energy source for microorganisms, supporting their growth and development (Waryanti, et al., 2013; Pitrianingsih, et al., 2014). Despite this, the bacterial count in the soil samples from Situ Cibinong was lower than in Situ Cibuntu. This discrepancy may be attributed to factors beyond organic carbon availability, such as toxic substances, competition with other microorganisms, and unfavorable environmental conditions, including temperature, pH, and humidity.

Environmental conditions, particularly soil pH, which was acidic in both samples, appear less conducive to microbial growth. Prior research indicates that highly acidic conditions can disrupt cellular metabolism and hinder microbial development (Larosa, et al., 2013). However, the impact of acidity varies among microbial species. In cases where pH levels fall outside the tolerance range of certain bacteria, cellular damage can occur, leading to inhibited growth (Larosa, et al., 2013).

Table 3. Soil Quality from Situ Cibuntu and Situ Cibinong.

Sampling Location		Temperature (°C)	pH	Moisture Content (%)	C-Organic Levels (%b/b)
Situ Cibuntu	1	28.50	5.07	3.23	8.36
	2	28.00	4.63	3.34	7.32
	3	28.30	4.60	3.41	8.01
	Average	**28.27**	**4.77**	**3.33**	**7.90**
	SD	**0.21**	**0.21**	**0.07**	**0.43**
Situ Cibinong	1	28.60	5.50	3.18	72.32
	2	28.50	5.43	6.14	162.49
	3	28.50	5.77	2.46	6.25
	Average	**28.53**	**5.57**	**3.93**	**80.35**
	SD	**0.05**	**0.15**	**1.59**	**64.04**

3.2 Bacterial Population Across Study Sites

The analysis of bacterial populations revealed that water and soil samples from Situ Cibuntu consistently exhibited higher Total Plate Count (TPC) values compared to those from Situ Cibinong (Table 4). In water samples, the TPC for Situ Cibuntu was ($7.5 \times 10^4 \pm 8.0 \times 10^4$) CFU/mL, whereas in Situ Cibinong, the TPC was ($2.5 \times 10^4 \pm 3.2 \times 10^4$) CFU/mL. Similarly, in soil samples, the bacterial count in Situ Cibuntu was significantly higher at ($170 \times 10^6 \pm 220 \times 10^6$) CFU/mL compared to ($7.2 \times 10^6 \pm 1.1 \times 10^6$) CFU/mL in Situ Cibinong.

Table 4. Number of Bacteria in Water and Soil Samples in Situ Cibuntu and Situ Cibinong.

Sampling Point		Water TPC (CFU/mL) Value	Soil TPC (CFU/mL) Value
Situ Cibuntu	1	3.7×10^4	1.1×10^7
	2	1.9×10^5	6.8×10^6
	3	5.8×10^2	4.8×10^8
	Average	**7.5×10^4**	**170×10^6**
	SD	**8.0×10^4**	**220×10^6**
Situ Cibinong	1	7.0×10^4	6.1×10^6
	2	4.0×10^3	8.3×10^6
	3	7.0×10^2	-
	Average	**2.5×10^4**	**7.2×10^6**
	SD	**3.2×10^4**	**1.1×10^6**

These findings indicate that the soil in Situ Cibuntu harbors a substantially more abundant bacterial population than that of Situ Cibinong. Variations in bacterial populations between the sites may be attributed to differences in environmental conditions, the chemical composition of water and soil, and anthropogenic activities. Situ Cibuntu, characterized by lush forest vegetation, experiences relatively cooler and more stable daytime water temperatures, which may facilitate microbial growth. Conversely, the absence of vegetation in Situ Cibinong likely results in greater diurnal temperature fluctuations, potentially hindering microbial development.

Vetiver root exudates play a critical role in supporting microbial activity by serving as carbon sources, while also influencing microbial community composition and driving riparian ecosystem processes such as nitrogen cycling and organic matter decomposition.

3.3 The Impact of Vetiver Root Extract on Bacterial Growth

The effect of vetiver root extract on the growth of heterotrophic bacteria in water and soil samples from the riverside areas of Situ Cibuntu and Situ Cibinong was investigated through a series of experiments. These experiments utilized culture media supplemented with varying concentrations of vetiver root extract, ranging from 0% to 100%. Bacterial density was assessed by measuring the optical density (OD) of the cultures following a 48-hour incubation period. The turbidity of liquid media, which correlates with bacterial population density, was quantified using a UV-Vis spectrophotometer at 600 nm. The absorbance value, which is proportional to the OD, reflects the abundance of bacterial cells in the solution (Seniati & Irham, 2019). Comparisons of bacterial density in water and soil samples from the two study locations are presented in Figure 1 and Figure 2.

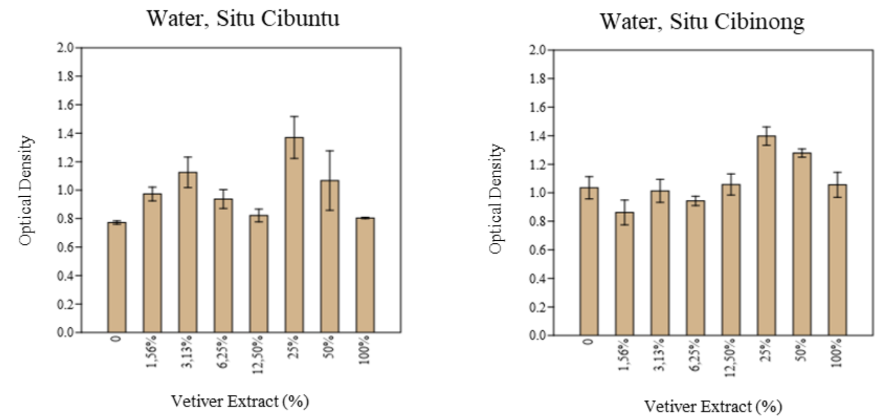

Figure 3. Bacterial density of the water sample.

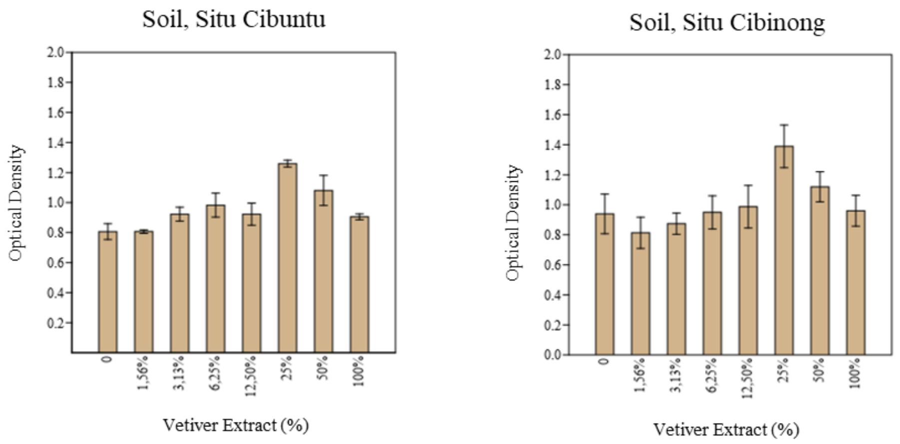

Figure 4. Bacterial density of the soil sample.

Bacterial density exhibited a decreasing trend as the concentration of vetiver root extract increased, with notable differences observed across the tested concentrations (Figure 3 and Figure 4). At extract concentrations between 0% and 25%, a significant increase in bacterial density was observed in both water and soil samples collected from Situ Cibuntu and Situ Cibinong. However, bacterial density began to decline at concentrations approaching 100%. The 25% extract concentration demonstrated the highest bacterial growth, significantly exceeding that observed at other concentrations in both aquatic and riparian soil bacteria (Table 7). The findings indicate that at lower concentrations (0-25%), the vetiver root extract likely contains organic compounds that act as readily available nutrients or growth stimulants for bacteria. In contrast, the extract exhibits antibacterial properties at higher concentrations (50-100%), thereby inhibiting bacterial growth. This dual response highlights the necessity for further investigation to identify the specific bioactive compounds present in vetiver root extract and elucidate their mechanisms of action on microbial communities.

Our results align with the study conducted by Narayanasamydamodaran, et al. (2025) which found Vetiver root exudates selectively shape the rhizosphere microbial community, favoring certain bacterial taxa that contribute to nutrient cycling and plant health. The abundance of beneficial groups like Proteobacteria and Actinobacteria, which are involved in nitrogen and phosphorus cycling, increases in the presence of specific exudate.

The release and diffusion of root exudates are affected by soil water content (Zhang, et al., 2023). Under optimal moisture, root exudates are more effectively distributed which in turn supports higher microbial activity. However, drought conditions can limit exudate diffusion and shift the balance of rhizosphere processes. This condition, in turn, makes water availability a more critical factor than exudation alone in sustaining microbial enzyme activities and nutrient cycling.

Table 7. Tukey's Pairwise Test

Comparison	P-Tukey's			
	Water, S. Cibuntu	**Water, S. Cibinong**	**Soil, S. Cibuntu**	**Soil, S. Cibinong**
0% - 25%	0,01534 *	0,03442 *	0,001122*	
1,56% - 25%		0,00118 *	0,00113*	0,04389*
1,56% - 50%		0,01209 *		
3,125% - 25%		0,02263 *	0,01652*	
6,25% - 25%		0,00572 *		
12,50% - 25%	0,02940 *		0,01678*	
25% - 100%	0,02332 *		0,01095*	

4. Conclusion

Significant variations in bacterial populations were observed between Situ Cibuntu and Situ Cibinong, emphasizing the influence of environmental factors, nutrient availability, and water quality parameters. Situ Cibinong exhibited higher bacterial densities, which were attributed to favorable environmental conditions characterized by elevated ammonium and organic carbon levels. In contrast, Situ Cibuntu showed greater bacterial abundance in both water and soil samples, potentially driven by higher concentrations of nitrate, organic nitrogen, and chemical oxygen demand (COD). Plant exudates act as a source of organic compounds in the riparian zone.

This study highlights the dual effects of vetiver root extract on bacterial growth, demonstrating that lower concentrations (0–25%) promote bacterial density, likely due to the presence of readily available nutrients, while higher concentrations (50–100%) inhibit bacterial proliferation through the action of antimicrobial compounds. This dynamic relationship is key to nutrient cycling, plant health, and ecosystem function in both natural and engineered environments, which in turn directly influence the structure and function of rhizosphere microbial communities. Water availability affects root exudation, thereby modulating microbial communities in the rhizosphere. These findings highlight the complex interplay between environmental conditions and vetiver root extract on bacterial communities. Further research is needed to identify specific compounds in vetiver root extract and their effects on bacteria in diverse aquatic ecosystems.

Data availability statement

The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request. Access requests will be considered to ensure compliance with ethical and legal guidelines.

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Conflict of interests

The authors declare no conflicts of interest related to this study. All efforts have been made to ensure that the research was conducted objectively and free from any commercial or financial relationships that could be construed as a potential conflict of interest.

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