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Profile of Phenolic Compounds and Phenol-Degrading Bacterial Colonies in Secondary Peat Forest Soil

Fiko Jervannes[1], Gusti Z. Anshari*[2], Evi Gusmayanti[3], Yulita Andriyani[4]

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[1,2,4]Department of Soil Science, Agriculture Faculty, Universitas Tanjungpura, Jalan Prof. Dr. Hadari Nawawi, Pontianak 78124, West Kalimantan, Indonesia
[3]Department of Agricultural Cultivation Budidaya Pertanian, Agriculture Faculty, Universitas Tanjungpura, Jalan Prof. Dr. Hadari Nawawi, Pontianak 78124, West Kalimantan, Indonesia
[3]Department of Agricultural Cultivation Budidaya Pertanian, Agriculture Faculty, Universitas Tanjungpura, Jalan Prof. Dr. Hadari Nawawi, Pontianak 78124, West Kalimantan, Indonesia
[2,3]Magister of Environmental Science, Universitas Tanjungpura, Jalan Prof. Dr. Hadari Nawawi Pontianak 78124, West Kalimantan, Indonesia

e-mail:
[1]c1051211077@student.untan.ac.id,
*[*2]gzanshari@live.untan.ac.id,*
[3]evi.gusmayanti@faperta.untan.ac.id,
[4]yulitaandriyani0@gmail.com

**Corresponding author*

Abstract. *Peat contains high levels of phenols and lignin, which are resistant to decomposition. Drainage canals lower the groundwater table, promoting microbial degradation. This study investigated microbial decomposition in a secondary peat swamp forest by quantifying phenol-degrading bacterial colonies and measuring phenol concentrations in peat soil from Malikian Village, Mempawah Regency, West Kalimantan. Samples were collected from three plots at two depths, the aerobic layer and the anaerobic layer, with fifteen (15) samples from each depth. Bacterial colonies were counted via mineral salt medium (MSM) with up to five dilutions, whereas phenolic compounds were measured via the Folin–Ciocalteu method. Paired t-tests revealed highly significant differences in both phenol concentration (p-value<0.001) and the number of phenol-degrading bacterial colonies (p-value<0.003) between the aerobic layer and the anaerobic layers. These findings indicate that peat decomposition is more pronounced in the aerobic surface layer than in the permanently waterlogged layer. This observation is attributed to the greater number of phenol-degrading bacterial colonies and lower phenol concentration in the surface layer than in the deeper layer. Consequently, the aerobic conditions in the surface layer of the secondary peat swamp forest facilitate accelerated peat decomposition.*

Keywords*: histosols, phenol-degrading bacteria, peat degradation*

Citation
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INTRODUCTION

Peat soils are formed from woody organic materials that typically contain high concentrations of aromatic compounds, including phenols, lignin, tannins, and humic acids. These aromatic compounds, characterized by their stable cyclic structures, play crucial roles in the accumulation of organic matter. Over time, this accumulation transforms tropical peat forests into substantial landscapes of carbon storage (Anshari et al., 2010, 2022). In anaerobic or low-oxygen conditions and acidic environments, the microbial activity responsible for the decomposition of peat-forming organic matter is significantly diminished, resulting in limited enzyme production for peat breakdown (Pind et al., 1994). Conversely, when the water table drops due to reduced rainfall or drainage, oxygen-rich conditions enhance phenol oxidase activity and accelerate peat decomposition (Morris and Waddington, 2011; Tolunay et al., 2024; Freeman et al., 2004).

Indonesia's drained and degraded peatlands are recognized as the world's largest source of carbon emissions, contributing approximately 668 million metric tons of carbon dioxide equivalent (CO_2-e) annually (Anshari et al., 2022; Hooijer et al., 2012; UNEP, 2022). These estimates are derived from greenhouse gas flux measurements, with water table depth commonly employed to assess peat decomposition rates (Asyhari et al., 2024; Deshmukh et al., 2021, 2020; Novita et al., 2022; Couwenberg and Hooijer, 2013; Hooijer et al., 2012; Miettinen et al., 2017; Page et al., 2011). However, decomposition is not solely driven by hydrological conditions but also by microbial activity (Kwon et al., 2013). Consequently, a comprehensive understanding of microbial processes is crucial for developing effective strategies to mitigate emissions from degraded peatlands.

Naturally, selected microbes, such as phenol-degrading bacteria and fungi, can degrade peats, converting organic matter into carbon emissions. Phenolic compounds, which play a crucial role in preventing peat degradation, are abundant in tropical peats. Phenols maintain a low acidity environment, inhibit decomposition enzymes, and reduce the availability of nitrogen for microbial growth. High concentrations of phenols decrease decomposition rates and support peat accumulation in waterlogged environments. Conversely, the breakdown of phenolic compounds accelerates peat degradation, leading to mineralization and the conversion of organic matter into CO_2.

The objective of this study was to establish a comparative analysis of phenolic compounds and phenol-degrading bacterial communities within the aerobic and anaerobic soil layers of a secondary peat forest ecosystem.

MATERIALS AND METHODS

Study Site and Duration

This research was conducted in Malikian Village, Mempawah Regency, over a period of five months from August to December 2024. The peatland conditions in the secondary forest at the study site had moderate vegetation density, and the soil was waterlogged. The research site map and peat sampling points are shown in Figure 1.

Field and laboratory Equipment

The tools used for sampling in this study included a Russian peat auger, ring sampler, PVC pipe, hoe, tape, camera, writing tools, machete, measuring knife, ziplock plastic bags, label paper, cling wrap, and aluminum foil. The equipment used for laboratory activities included an analytical balance, Petri dishes, test tubes, micropipettes, pipette tips, a laminar flow cabinet, a beaker glass, a bunsen burner, measuring cylinders, an autoclave, an oven, a pH meter, a spectrophotometer, vial bottles, a centrifuge, a shaker, a mortar, an Erlenmeyer flask, cotton, tissue, face masks, aluminum foil, and cling wrap.

Jurnal Biodjati 10(2): 352–360, November 2025

JURNAL BIODJATI

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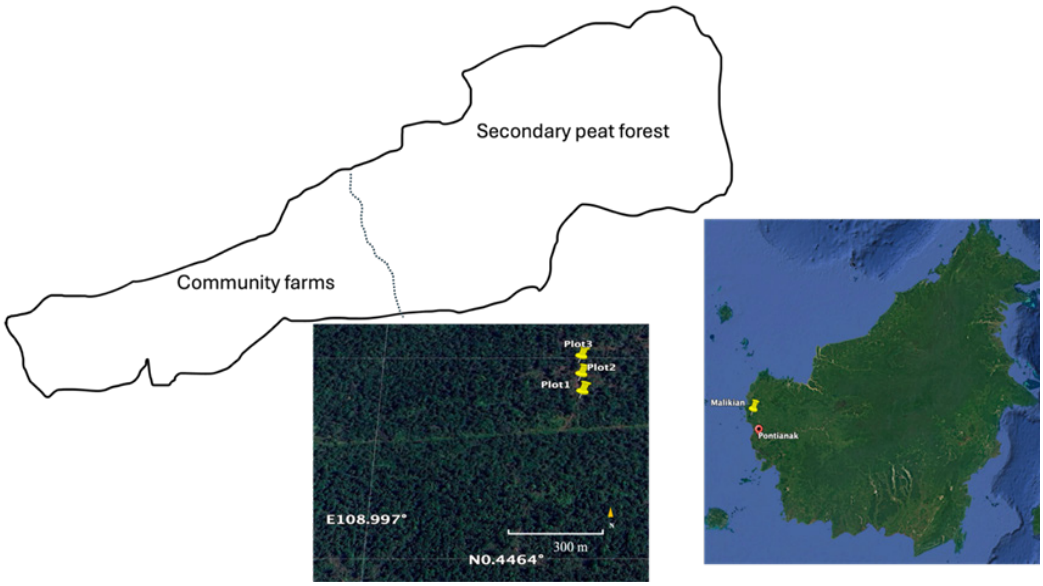

Figure 1. A map of the study site, showing Malikian Village in top panel and sample plots in the bottom panel. The location of Malikian village is approximately 115 km from Pontianak, the capital city of West Kalimantan Province.

Sampling Procedure

In this study, a survey method was employed. Sampling was conducted in three plots, spaced 40 cm apart and aligned along a single transect line (as depicted in Figure 1). Peat samples were collected using a Russian peat auger at depths of 0-50 cm (representing the aerobic layer) and 200-250 cm (the anaerobic layer). Samples for phenol compound concentration, pH, gravimetric water content, and bulk density analyses were separately collected from the bacterial samples. Weekly samples of total plate counts were taken for a single plot to ensure the uncontaminated nature of the samples.

The number of samples used for phenol-degrading bacteria testing, phenol, pH, Bulk Density (BD), and Gravimetric Water Content (GWC) analyses was 15 samples for each layer. The total sample numbers in both aerobic and anaerobic layers were 30 samples.

The bacterial test samples were stored in amber-colored glass bottles with lids at ambient temperature without the use of ice packs. The samples were kept out of direct sunlight and were immediately transported to the Laboratory of Plant Pests and Diseases, Faculty of Agriculture, Universitas Tanjungpura, where phenol-degrading bacterial colony tests were conducted. The soil acidity was measured using a pH meter with a 1:5 ratio of sample to distilled water. Samples of GWC and BD were dried in an oven at 70°C until they reached a constant dry weight. BD represents the weight of the constant dry sample in grams per sample volume in cubic centimeters. GWC represents the amount of water per the weight of the constant dry sample.

Laboratory Analysis

The total plate count (TPC) medium used to grow phenol-degrading bacteria was mineral salt medium (MSM) (Hatakeyama et al., 2016; Sabri et al., 2025; Zhang et al., 2023), which was prepared in advance of the sampling. Table 1 presents the list of chemical components of the MSM, which were sterilized by autoclaving at 121°C for 15 minutes. Pure phenol was added to the medium when the temperature reached approximately 40°C. Phenol served as the carbon source for the phenol-degrading bacteria studied.

Bacterial isolation was conducted in duplicate as technical replicates via the pour

Jurnal Biodjati 10(2): 352–360, November 2025

JURNAL BIODJATI

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Table 1. Composition of mineral salt medium (MSM)

Nama Senyawa	Formula	Concentration (g/L)
Magnesium sulfate heptahydrate	$MgSO_4$. 7H2O	0.2
Potassium dihydrogen phosphate	KH_2PO_4	1
Calcium chloride	$CaCl_2$	1
Dipotassium dihydrogen phosphate	K_2HPO_4	1
Ammonium nitrate	NH_4NO_3	1
Ferrous cholride/Iron (III) cholride	$FeCl_2$	0.05
Sodium chloride (>99,5%)	NaCl	8.5
Agar		18
Pure Phenol (99%)	C_6H_5OH	0.6

plate method. One gram of peat soil sample was placed into a test tube containing 9 ml of sterile physiological saline solution (NaCl) and homogenized at a 10^{-1} dilution. The sample was then serially diluted to 10^{-5}. The total plate count (TPC) samples were incubated in an incubator at 24–27 °C for 5 days to observe and count the bacterial colonies that developed. Colony counts were conducted in the range of 30–300 CFU/g and reported as the mean of the duplicates.

The total phenol test was performed using 1 gram of a fresh soil sample, which was dissolved in 50 mL of methanol and ground in a porcelain mortar. The sample was macerated for 1 hour on a shaker at 200 rpm and then centrifuged for 3 minutes at 3500 rpm. For measurement, 0.2 mL of the supernatant was transferred to a test tube, followed by the addition of 1 mL of 10% Folin–Ciocalteu reagent and 3 mL of 2% Na_2CO_3. The mixture was incubated in the dark for 30 minutes. The absorbance was measured at a wavelength of 765 nm via a UV–Vis spectrophotometer (Lim et al., 2017). Measurements of phenolic compounds, pH, gravimetric water content, and bulk density were conducted at the Soil Quality Laboratory, Faculty of Agriculture, Universitas Tanjungpura.

Data Analysis

Data analysis was carried out via paired t-tests, the Real Statistics Resource Pack add-in for Microsoft Excel" (https://real-statistics.com, accessed May 20, 2025) to compare total phenol-degrading bacterial colony counts and total phenolic compound concentrations between the aerobic and anaerobic peat layers. Paired t-tests were also applied to the pH, water content, and bulk density data. The peat maturity level was assessed via the von Post scale at the time of sampling, and Spearman's correlation was used to examine the relationship between phenolic compound concentrations and bacterial colony counts.

RESULTS AND DISCUSSION

Like other peat soils, the peat at the study plot is acidic, has a low bulk density, and a high water content (Table 2). A paired t-test revealed that the pH value in the anaerobic layer was significantly different from that in the aerobic layer (t-statistic=2.14, p-value<0.003). The average bulk density and moisture content did not differ significantly. The high moisture content in both the upper and lower layers indicates that the conditions were saturated at the time of sampling.

The peat maturity level, as determined by the von Post test, was high in the upper layer and partially fibric in the lower layer. Fibric peat layers are known to have high moisture contents but lower water-holding capacities than hemic and sapric peat layers.

Table 2. Results of paired tests on pH, bulk density (BD), and gravimetric water content (GWC)

Statistics	pH		BD (g cm^{-3})		GWC (%)	
	Aerobic Layer	Anaerobic Layer	Aerobic Layer	Anaerobic Layer	Aerobic Layer	Anaerobic Layer
Mean	3.75a	3.86b	0.09a	0.08a	860a	953a
Standard error	0.02	0.03	0.00	0.00	30	32
Median	3.74	3.88	0.09	0.08	865	999
Standard deviation	0.07	0.10	0.01	0.01	117	123
Sample variance	0.00	0.01	0.00	0.00	13,596	15,251
Maximum	3.87	3.99	0.12	0.11	1,055	1,111
Minimum	3.64	3.68	0.07	0.07	611	702

Note: The same lowercase letter used to represent mean values indicates a statistically insignificant difference between the aerobic and anaerobic layers, as determined by a paired t-test at a 5% significance level. BD = Bulk Density; GWC = Gravimetric Water Content.

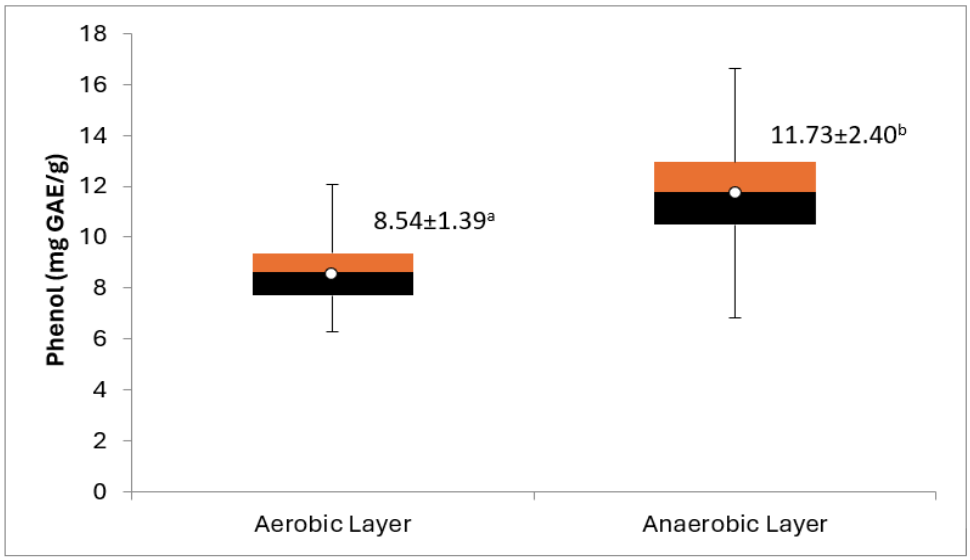

Figure 2. The box plot of phenolic compound concentrations revealed a highly significant difference between the aerobic and anaerobic layers (t-stat=2.14; p value <0.001).

Concentration of phenolic compounds

Figure 2 shows the results of phenolic compound measurements in the aerobic and anaerobic layers, which exhibit significant differences. The relatively lower concentration of phenolic compounds in the aerobic layer suggests that the rate of peat decomposition in this layer is higher than in the anaerobic layer (Yule et al., 2016). Freeman et al. (2004) reported that when peat conditions become aerobic or oxygen-rich, bacterial activity increases, leading to the release of the enzyme phenol oxidase, which plays a role in breaking down phenolic compounds (Pind et al., 1994). This decomposition process is intensive and continues at varying speeds. When peat is waterlogged and anaerobic, the decomposition rate may initially slow, but it can rapidly increase once the conditions shift to aerobic. Further research is needed to understand better the dynamics of phenolic compound concentrations in relation to the rate of peat decomposition.

Jurnal Biodjati 10(2): 352–360, November 2025

JURNAL BIODJATI

http://journal.uinsgd.ac.id/index.php/biodjati

Phenol-degrading bacterial colonies

The profile of the phenol-degrading bacterial colonies is shown in the box plot in Figure 3. The mean colony count was significantly higher in the aerobic layer compared to the anaerobic layer. Spearman's correlation test between the number of bacterial colonies and phenolic compound concentration yielded a rho value of -0.41 (t-statistic=-2.34; p-value<0.03), indicating that higher abundances of phenol-degrading bacteria are associated with lower concentrations of phenolic compounds. The scatter plot in Figure 4 illustrates the relationship between the number of phenol-degrading bacterial colonies and the concentration of phenolic compounds.

The phenolic compound concentrations were greater in the anaerobic layer than in the aerobic layer—permanent anaerobic conditions in the lower layer limit bacterial activity in decomposing organic matter in peat soil. In contrast, the dynamic aerobic conditions in the upper layer support accelerated decomposition of the peat. This phenomenon is likely triggered by the release of phenol oxidase enzymes, which play a role in breaking down phenolic compounds into simpler organic compounds that are more easily decomposed biologically (Freeman et al., 2004; Kim et al., 2024; Sinsabaugh, 2010).

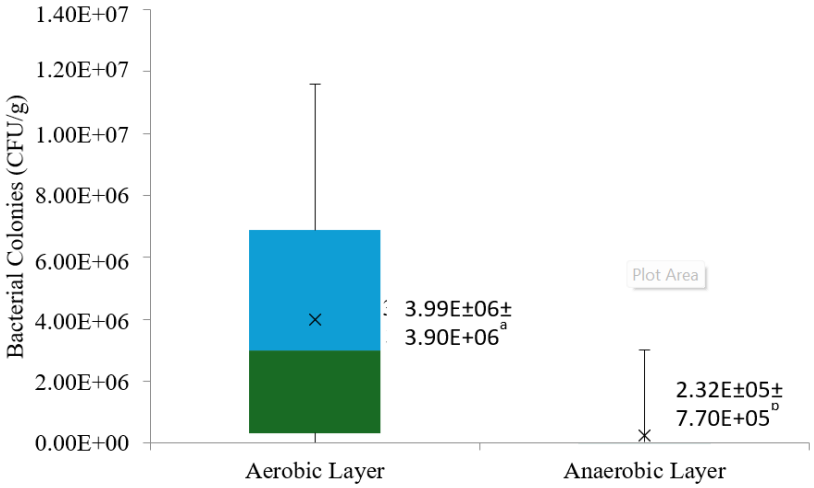

Figure 3. The comparison of phenol-degrading bacterial colonies between the aerobic layer (0–25 cm) and the anaerobic layer (200–225 cm) revealed a statistically significant difference (t-statistic = 2.14; p value<0.003).

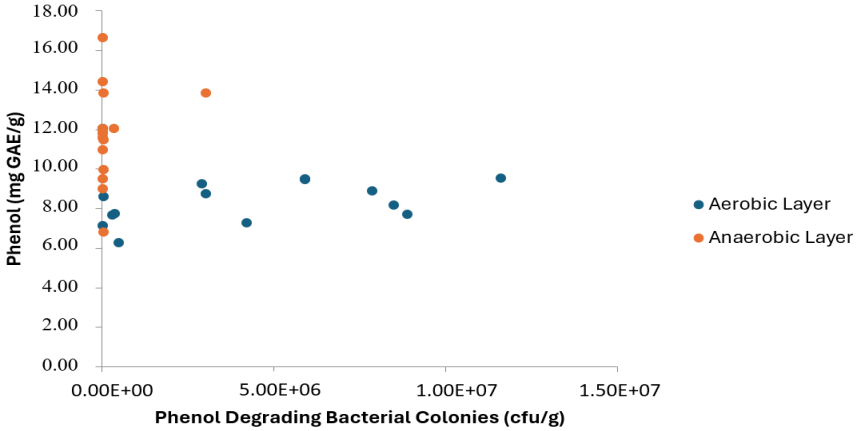

Figure 4. A negative correlation was detected between the number of phenol-degrading bacterial colonies and the concentration of phenolic compounds

CONCLUSION

The study findings revealed a substantial disparity in the count of phenol-degrading bacterial colonies between the aerobic layer (1.2×10^7 CFU/g) and the anaerobic layer (3.0×10^2 CFU/g). Conversely, the concentration of phenolic compounds was greater in the anaerobic layer. The negative correlation between the phenolic compound concentration and the number of phenol-degrading bacterial colonies suggests that the decomposition rate of peat is greater in the aerobic upper layer than in the anaerobic lower layer. Phenolic compounds tend to remain stable in oxygen-depleted conditions due to the inhibition of microbial activity, which restricts the oxidation of complex aromatic compounds present in the organic matter that constitutes peat.

AUTHOR CONTRIBUTION

F.J. drafted a research proposal for his undergraduate thesis, conducted sample campaigns, and examined and analyzed samples. **G.A.** and **E.G.** formulated the research question, monitored the fieldwork, supervised the laboratory work, guided the data analysis, and revised the manuscript. **Y.A.** collected the samples and conducted the laboratory experiments.

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CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

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