Physicochemical Variation of Water, Diversity, and Bacteria Community Structure in Post-coal Mining Ponds of Different Ages in Samarinda, East Kalimantan, Indonesia

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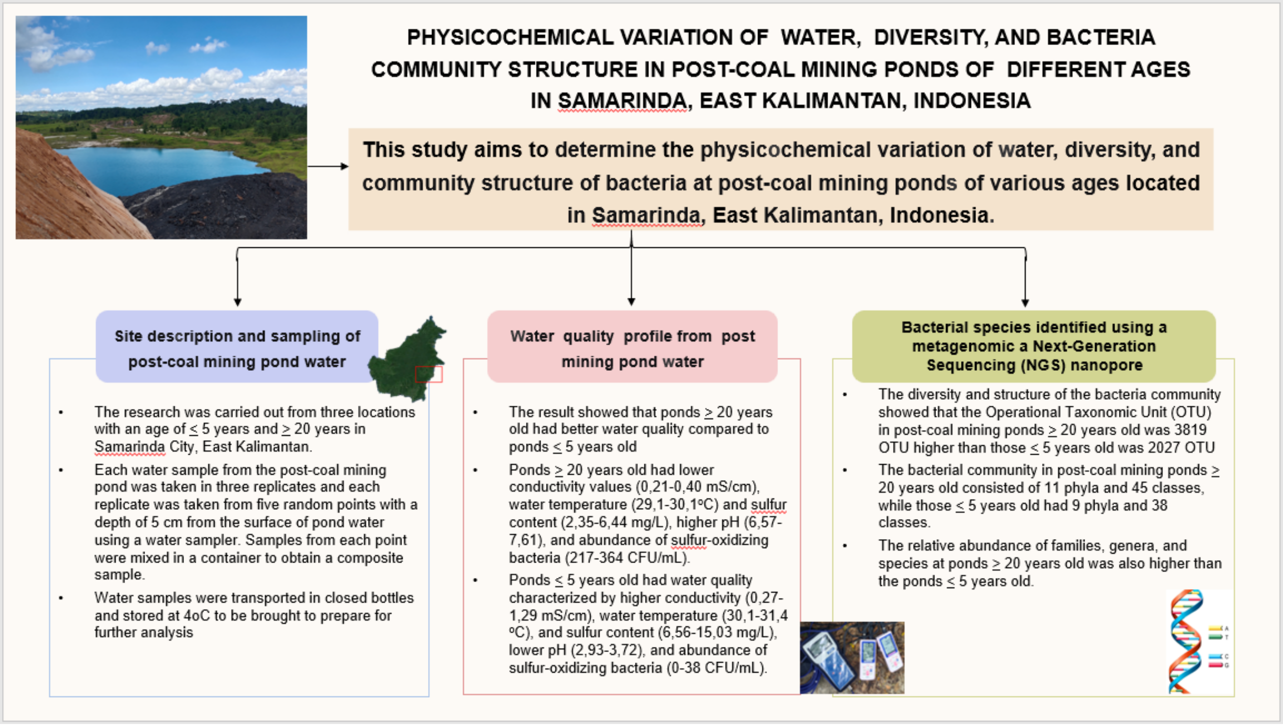
GRAPHICAL ABSTRACT



PHYSICOCHEMICAL VARIATION OF WATER, DIVERSITY, AND BACTERIA COMMUNITY STRUCTURE IN POST-COAL MINING PONDS OF DIFFERENT AGES IN SAMARINDA, EAST KALIMANTAN, INDONESIA

This study aims to determine the physicochemical variation of water, diversity, and community structure of bacteria at post-coal mining ponds of various ages located in Samarinda, East Kalimantan, Indonesia.

Site description and sampling of post-coal mining pond water

- The research was carried out from three locations with an age of ≤ 5 years and ≥ 20 years in Samarinda City, East Kalimantan.
- Each water sample from the post-coal mining pond was taken in three replicates and each replicate was taken from five random points with a depth of 5 cm from the surface of pond water using a water sampler. Samples from each point were mixed in a container to obtain a composite sample.
- Water samples were transported in closed bottles and stored at 4oC to be brought to prepare for further analysis

Water quality profile from post mining pond water

- The result showed that ponds ≥ 20 years old had better water quality compared to ponds ≤ 5 years old
- Ponds ≥ 20 years old had lower conductivity values (0,21-0,40 mS/cm), water temperature (29,1-30,1°C) and sulfur content (2,35-6,44 mg/L), higher pH (6,57-7,61), and abundance of sulfur-oxidizing bacteria (217-364 CFU/mL).
- Ponds ≤ 5 years old had water quality characterized by higher conductivity (0,27-1,29 mS/cm), water temperature (30,1-31,4 °C), and sulfur content (6,56-15,03 mg/L), lower pH (2,93-3,72), and abundance of sulfur-oxidizing bacteria (0-38 CFU/mL).

Bacterial species identified using a metagenomic a Next-Generation Sequencing (NGS) nanopore

- The diversity and structure of the bacteria community showed that the Operational Taxonomic Unit (OTU) in post-coal mining ponds ≥ 20 years old was 3819 OTU higher than those ≤ 5 years old was 2027 OTU
- The bacterial community in post-coal mining ponds ≥ 20 years old consisted of 11 phyla and 45 classes, while those ≤ 5 years old had 9 phyla and 38 classes.
- The relative abundance of families, genera, and species at ponds ≥ 20 years old was also higher than the ponds ≤ 5 years old.

ABSTRACT

This study aims to determine physicochemical variation of water, diversity, and community structure of bacteria at post-coal mining ponds of various ages located in Samarinda, East Kalimantan, Indonesia. Bacteria species were identified using a metagenomic approach on the Next-Generation Sequencing (NGS) nanopore platform and analyzed using the QIIME2 pipeline. The result showed that ponds aged ≥ 20 years old had better water quality compared to others ≤ 5 years old. In addition, ponds ≥ 20 years old had lower conductivity values (0,21-0,40 mS/cm), water temperature (29,1-30,1°C), and sulfur content (2,35-6,44 mg/L), as well as higher pH (6,57-7,61) and abundance of sulfur-oxidizing bacteria (SOB) (217-364 CFU/mL). Based on the results, ponds ≤ 5 years old had water quality characterized by higher conductivity (0,27-1,29 mS/cm), water temperature (30,1-31,4 °C), and sulfur content (6,56-15,03 mg/L), lower pH (2,93-3,72), and abundance of SOB (0-38 CFU/mL). Diversity and structure of bacteria community showed that the Operational Taxonomic Unit (OTU) in post-coal mining ponds ≥ 20 years old was 3819 compared to 2027 in those ≤ 5 years old. Bacteria community in post-coal mining ponds ≥ 20 years old consisted of 11 phyla and 45 classes, while those ≤ 5 years old had 9 phyla and 38 classes. Moreover, the relative abundance of families, genera, and species at ponds ≥ 20 years old was also higher. The high water quality, diversity, and community structure of bacteria at ponds ≥ 20 years old showed the successful remediation of ponds water by SOB.

Keywords: open-cut coal mining, coal mine, bacteria community, pond age, water quality

1. Introduction

East Kalimantan is one of the provinces in Indonesia with abundant coal mining potential due to the widespread availability of resources. In 2019, the Regional Government of East Kalimantan Province issued a total of 386 coal Mining Business Permits (IUP). These permits were distributed across various regions, with 171 in Kutai Kartanegara Regency, 27 in Paser Regency, 23 in Berau Regency, 77 in West Kutai Regency, 33 in East Kutai Regency, 18 in North Penajam Paser Regency, and 37 in Samarinda City (Dinas Pertambangan dan Energi Kalimantan Timur, 2021). In addition, Samarinda City is widely known to be the second-largest coal-producing region in East Kalimantan with a flat and hilly topography between 10-200 meters above sea level as well as a land area of 718 km^2. In the 2000s, the development of coal production in Samarinda City increased, leading to its consideration as mining city because approximately 38,814 Ha (54%) of the total 71,823 Ha was used as coal mining areas (Azwari & Rajab, 2021).

In line with previous studies, coal mining companies often implement the open-cut mining system (Jiayin et al., 2020; Park et al., 2020). This system typically comprises various stages, namely cleaning the surface of the soil, stripping the overburden, excavating the soil layer or coal seam, and transporting coal to the stockyard for further use (Park et al., 2020). However, the open pit mining system often causes the formation of large openings, which are filled with water containing sulfur (Koščová et al., 2018) and become a habitat for bacteria. The sulfur contained in post-coal mining pool water is obtained from coal containing 2-11% sulfur as well as coal-washing process. During washing, the elements present in coal dissolve or are subjected to several chemical reactions and physical reactions. This causes the washing products to dissolve into the washing solution (Dutta et al., 2018). One of the groups of bacteria species present in post-coal mining ponds is sulfur-oxidizing bacteria (SOB).

According to several reports, SOB play an essential role in the oxidation process of H_2S and other reduced inorganic sulfur elements (elemental sulfur (S0), pyrite (FeS_2), and sulfate (SO_2)). This is primarily because these elements can function as an energy source and sulfur source for bacteria metabolism (Hidayat et al., 2017). Various studies (Pourbabaee et al., 2020 and Rana et al., 2020) have shown that SOB is a group of bacteria with bioremediation abilities by carrying out sulfur oxidation. Sulfur is an essential element for life, which is typically found in 2 amino acids and often binds to hydrogen and oxygen ions in water. The element is often found in water in the form of sulfide (S_2^-), hydrogen sulfide (H_2S), ferrous sulfide (FeS), sulfur dioxide (SO_2), sulfite (SO_3), and sulfate (SO_4). The combination of sulfur and hydrogen has been shown to lead to the formation of sulfuric acid. Another study showed that sulfur is often found in combination with alkali metals in lakes and rivers. Despite the potential of SOB, their activity is influenced by environmental factors that drive sulfur cycle (Méndez-García et al., 2015; Zhang et al., 2017) and age of post-coal mining ponds, where the older pond, the higher diversity of life (Tala'ohu & Irawan, 2014). Post-mining ponds generally consist of young and old variants aged < 5 years old and > 20 years old, respectively (Prasetiyono, 2015). Therefore, this study aims to 1) determine bacteria community structure isolated from post-coal mining ponds water of various ages in Samarinda, and 2) assess the variations in water quality profiles and the abundance of SOB in post-coal mining ponds aged ≤ 5 years and ≥ 20 years old. The results are expected to provide new insights into indigenous bacteria species in post-coal mining ponds water ecosystems in Samarinda City, East Kalimantan, Indonesia, the correlation model among physicochemical parameters, and the density of SOB of ponds.

2. Materials and methods

2.1. Site description and water sampling of post-coal mining ponds water

This study was carried out from July-September 2022 in 3 locations of post-coal mining ponds with age of ≤ 5 years and 3 locations of post-coal mining ponds with age of ≥ 20 years in Samarinda City, East Kalimantan. The procedures were also conducted at Water Quality Laboratory of the Faculty of Fisheries and Marine Sciences, Mulawarman University, Samarinda, Indonesia, and the Animal Ecology and Diversity Laboratory, Faculty of Mathematics and Natural Sciences, Universitas Brawijaya, Malang, Indonesia. The details of the sampling locations are presented in Figure 1. Each water sample from post-coal mining ponds was taken in 3 replicates and each replicate was obtained from 5 random points with a depth of 5 cm from the surface of ponds water using water sampler. Samples from each point were mixed in a container to obtain a composite sample. This procedure was repeated with samples from every 3 replicates and 5 different points. For further analysis, water samples were transported in sealed bottles, stored at 4 ºC, and transported to the Microbiology Laboratory, Faculty of Mathematics and Natural Sciences, Universitas Brawijaya, Malang, Indonesia.

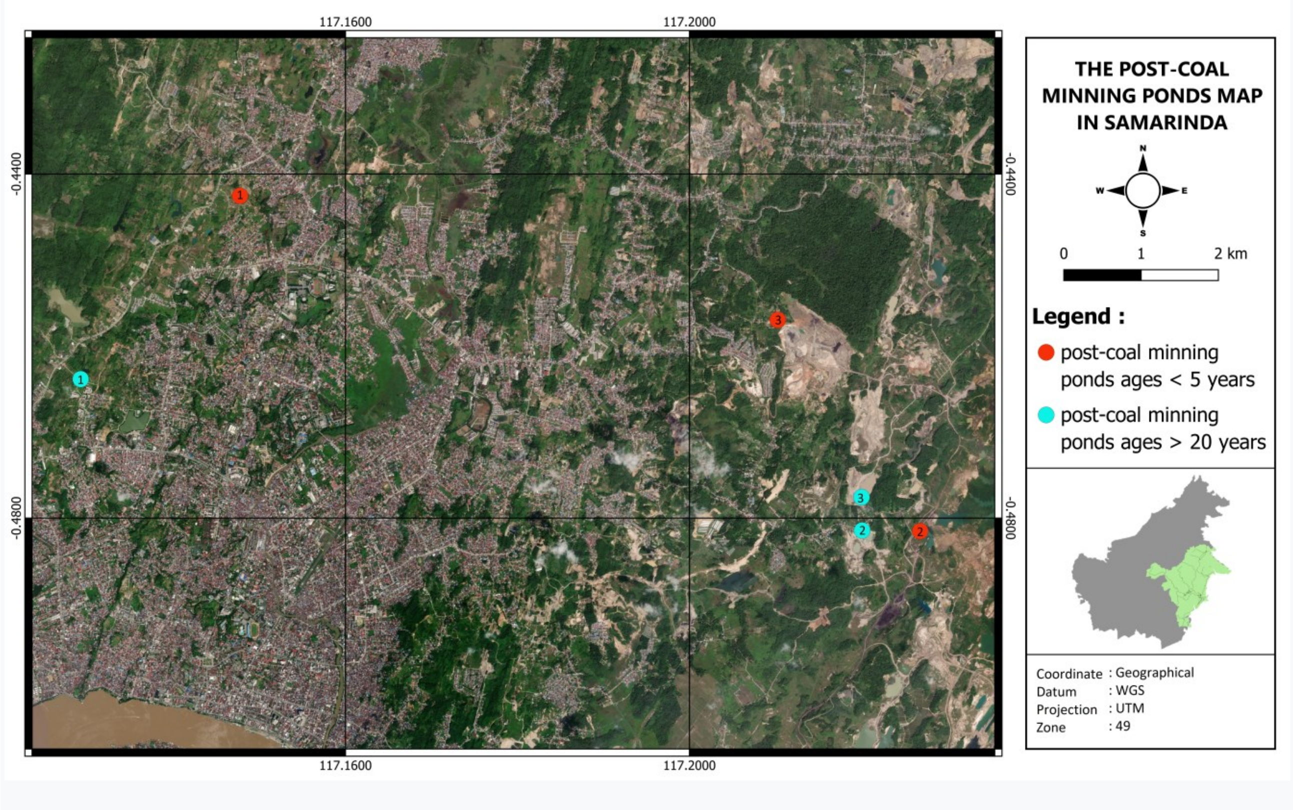


Figure 1. Details of the sampling locations

2.2. Analysis of physicochemical and SOB communities structure of water ponds

Physicochemical parameters of water ponds were analyzed in-situ (direct) and ex-situ (in the laboratory) to determine water quality of age of both post-coal mining. A total of 1 liter of water sample was taken from each pond (Yousef et al., 2019; Ma et al., 2020). Water parameters analyzed in situ were temperature, pH, and dissolved oxygen, while those analyzed in the laboratory were COD (SNI 6989.2:2019), BOD (APHA,5210-B,22ND th.2012), and SO_4 (SNI 6989.20-2009).

In addition, all ponds water samples of the same age were mixed into 1 sample. The mixed ponds water was filtered using a filter membrane (Merck) with a series of pore diameters of 11.0 μm, 0.45 μm, and 0.20 μm. The 0.20 μm filter membrane containing bacteria cells was cut into small pieces and placed into a Lysing Matrix E tube. Bacteria DNA was extracted according to the FastDNA Spin Kit (MPBIO) Germany protocol with modifications, namely 1) increasing the homogenization time of the sodium phosphate buffer sample from 5 to 10 seconds using a vortex, 2) the suspension was incubated for 10 minutes at room temperature (18 ± 1 °C) after which the supernatant protein precipitate solution was inverted. The increase in homogenization time was intended to ensure that the sample and sodium phosphate buffer were well mixed, while incubation of the supernatant-precipitate solution was performed for complete precipitation of protein-supernatant.

2.3. Amplification of bacteria chromosomal 16S rDNA

Extracted DNA was analyzed qualitatively by 1% agarose gel electrophoresis. The DNA concentration and purity were measured using a NanoDrop Spectrophotometer. In addition, amplification of bacteria chromosomal 16S rDNA was carried out through Oxford Nanopore Technology (ONT) 16S ribosomal RNA (16S rRNA) gene sequencing, which provided cover for the full 16S rRNA gene sequence (region V1–V9). Nanopore sequencing was operated by MinKNOW software version 22.05.7. Base-calling was carried out using Guppy version 6.1.5, FASTQ file quality was visualized using NanoPlot, and quality filtering was performed using NanoFilt.

2.4. Taxa richness and diversity analysis

The alpha diversity and beta diversity were subsequently performed using the normalized data. Alpha diversity was applied to analyse species diversity in a sample using observed-species, Chao1, Shannon, Simpson, and ACE expansion with QIIME (Version 1.7.0). In addition, community richness was identified using Chao1, ACE, Shannon, and Simpson indexes. Community diversity was identified using the Shannon and Simpson indexes to assess species richness and diversity for each water sample from different locations (Wang et al., 2018).

2.5. Physicochemical data of water analysis

Physicochemical data of water at post-coal mining ponds was analyzed according to analysis of variance and biplot analysis using Principal Component Analysis (PCA) (Souza et al., 2021; Wang et al., 2018) with the PAST program. Differences in water quality between post-mining ponds were identified by ANOVA followed by the Tukey HSD test (BOD and SOB) (Sheng et al., 2023). The analysis was continued with Brown Forsythe, Games-Howell test (pH, DO, conductivity, temperature, TSS, and Sulfate), Kruskal Wallis, and Mann Whitney test (COD) (Reddy et al., 2020) using SPSS v.22. The correlation among physicochemical parameters and abundance of SOB of post-coal mining ponds water were determined by Pearson correlation analysis (Wang et al., 2018) using SPSS v.22.

3. Results and Discussion

3.1. Water quality profile from post-mining ponds water in Samarinda

Physicochemical and the abundance of SOB in water at post-coal mining ponds ≤ 5 years and ≥ 20 years old are presented in Table 1.

Table 1. Physicochemical and SOB parameters of post-coal mining ponds water in Samarinda

No	Parameter	Post-coal Mining Ponds					
		≤ 5 years old			≥ 20 years old		
		1	2	3	1	2	3
1	pH	$2{,}93^a$	$3{,}23^b$	$3{,}72^c$	$6{,}57^d$	$6{,}84^{de}$	$7{,}61^e$
2	DO (ppm)	$3{,}56^a$	$3{,}71^a$	$3{,}72^a$	$3{,}12^a$	$3{,}27^a$	$3{,}56^a$
3	Conductivity (S/m)	$1{,}29^e$	$0{,}56^d$	$0{,}40^c$	$0{,}27^b$	$0{,}23^{ab}$	$0{,}21^a$
4	Temperature (ºC)	$30{,}10^{ab}$	$30{,}43^b$	$31{,}40^c$	$29{,}60^a$	$29{,}13^{abc}$	$30{,}10^{ab}$
5	TSS (mg/L)	$0{,}81^a$	$0{,}05^a$	$0{,}24^a$	$0{,}12^a$	$0{,}98^a$	$0{,}86^a$
6	COD (mg/L)	$16{,}20^a$	$15{,}15^a$	$13{,}12^a$	$18{,}14^{ab}$	$23{,}98^b$	$24{,}33^b$
7	BOD (mg/L)	$6{,}40^a$	$6{,}07^a$	$4{,}55^a$	$5{,}54^a$	$5{,}82^a$	$7{,}67^a$
8	Sulfate (mg/L)	$15{,}03^{ab}$	$9{,}50^{ab}$	$6{,}56^{ab}$	$6{,}44^{ab}$	$4{,}74^b$	$2{,}35^a$
9	Abundance of SOB (CFU/mL)	0^a	30^a	38^a	259^b	217^b	364^c

Note: numbers followed by the same letter in the same parameter are not significantly different based on the 5% ANOVA test.

Table 1 showed that ponds ≥ 20 years old were characterized by lower conductivity (0,21-0,40 mS/cm), water temperature (29,1-30,1 ºC), sulfur content (2,35-6,44 mg/L), higher pH (6,57-7,61), and abundance of SOB (217-364 CFU/mL). Meanwhile, ponds ≤ 5 years old had lower water quality, higher conductivity (0,27-1,29 mS/cm), water temperature (30,1-31,4 ºC), and sulfur content (6,56-15,03 mg/L), lower pH (2,93-3,72), and an abundance of SOB (0-38 CFU/mL). Tomal (2020) stated that the growth and development of microorganisms were stimulated or inhibited by environmental factors. The main factors affecting bacteria growth included nutrients, pH, water, oxygen (Chrismanuel et al., 2012), temperature, and humidity in the environment (Tomal, 2020).

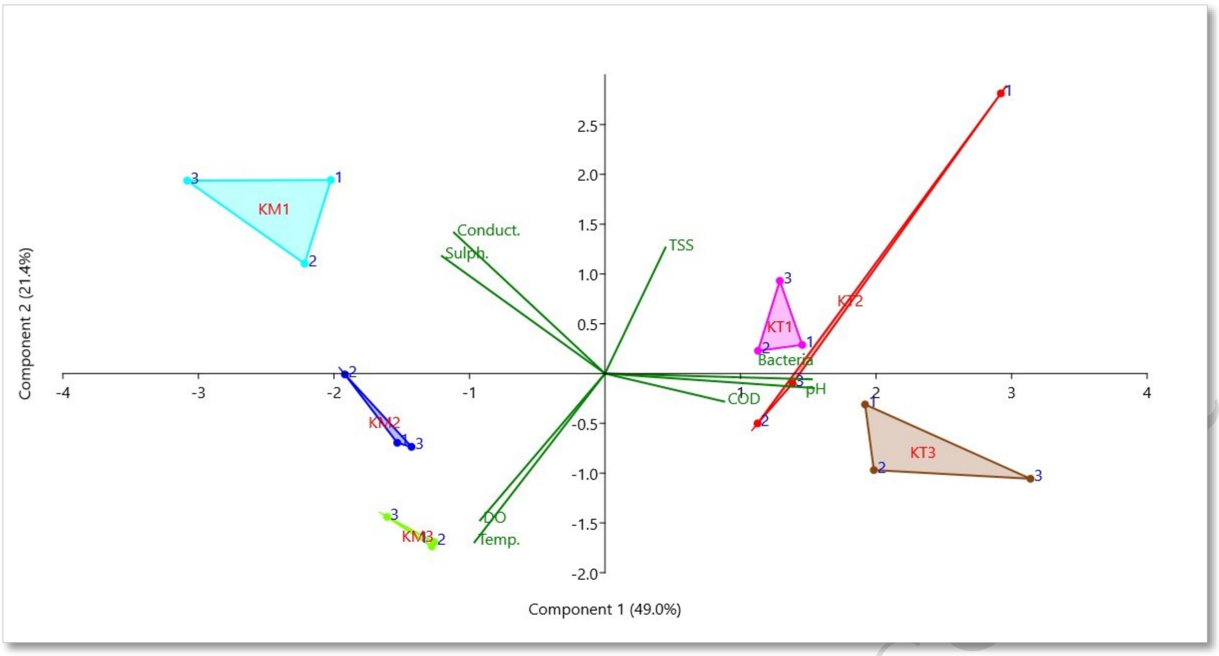
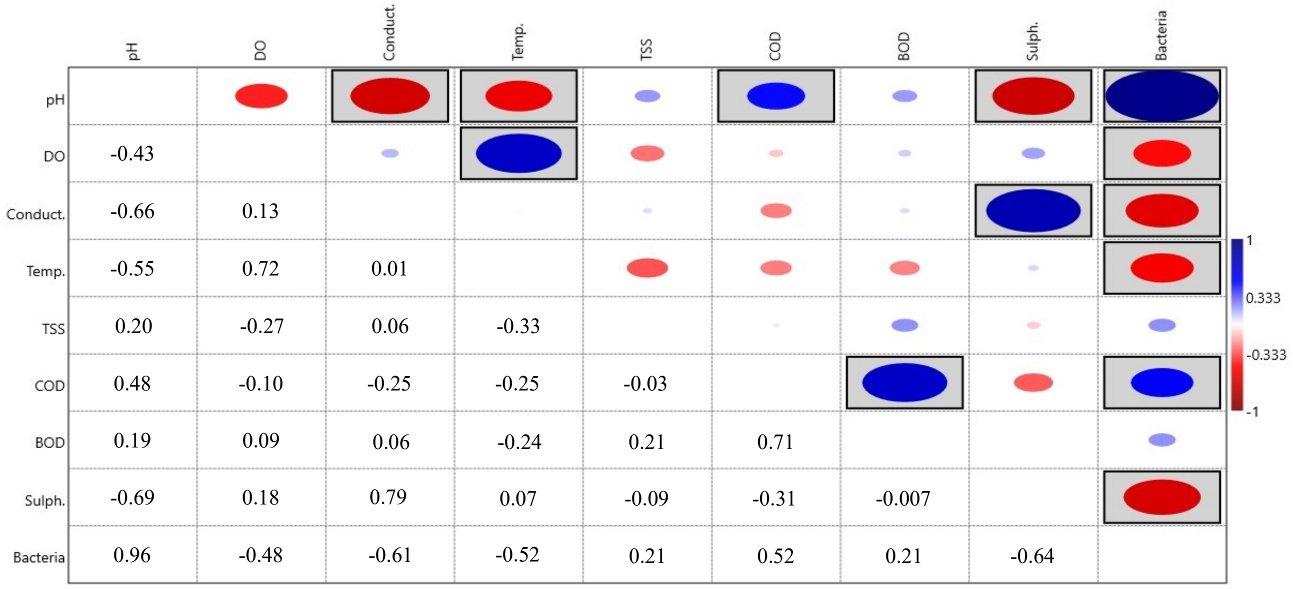


Figure 2. Loading plot post-coal mining ponds water in Samarinda based on biplot analysis using PCA; KM1, KM2, KM3 = post-coal mining ponds ≤ 5 years old; KT1, KT2, KT3 = post-coal mining ponds ≥ 20 years old

Figure 2 showed that the parameters of ponds ≤ 5 and ≥ 20 years old were in one group. The results showed that there was a significant difference in water quality between the 2 types of ponds. Water of ponds ≤ 5 years old was characterized by high conductivity, sulfur content, temperature, and low of density of SOB, COD, and pH. Meanwhile, water of ponds ≥ 20 years old was characterized by high TSS, density of SOB, pH, and COD, as well as low conductivity, sulfur content, DO, and temperature. The higher SOB density in ponds ≥ 20 years old led to lower sulfur concentration compared to those aged ≤ 5 years old. This was because ponds ≥ 20 years old had a more complex and diverse microorganism community that developed and formed a stable ecosystem. The thriving microorganism community could have SOB species that had adapted and developed well under ponds conditions. Stable environmental conditions could support the growth and activity of SOB more effectively, leading to higher population densities and the accumulation of sulfur and other compounds necessary for the growth of SOB (Rawlings, 2005).



	pH	DO	Conduct.	Temp.	TSS	COD	BOD	Sulph.	Bacteria
pH									
DO	-0.43								
Conduct.	-0.66	0.13							
Temp.	-0.55	0.72	0.01						
TSS	0.20	-0.27	0.06	-0.33					
COD	0.48	-0.10	-0.25	-0.25	-0.03				
BOD	0.19	0.09	0.06	-0.24	0.21	0.71			
Sulph.	-0.69	0.18	0.79	0.07	-0.09	-0.31	-0.007		
Bacteria	0.96	-0.48	-0.61	-0.52	0.21	0.52	0.21	-0.64	

Figure 3. Correlation Pearson between physicochemical parameters and SOB density of water

Correlation Pearson analysis was a method for analyzing the relationship between variables of water ponds. When there was a relationship, the changes that occurred in one of the variables led to changes in the other variables. The term was said to be a causal term, and it was the hallmark of correlation analysis. The results of the study in Figure 3 showed there was a positive correlation/relationship with SOB. This showed that an increase in the density of SOB caused an increment in pH and COD values, with pH value affecting the microbial metabolism. The gene expression in *Sulfobacillus thermosulfidooxidans* and *Ferroplasma thermophilum* was primarily affected by pH values (Peng et al., 2019). Maintenance of pH was important due to its significant effect on bacteria growth. In addition, bacteria growth was determined by optimizing pH and incubation time. The optimization of environmental conditions was very important for the enhancement of bacteria growth and for designing an effective biodegradation strategy. The level of the relationship was significant because it was more than 0.5. Figure 3 also showed the negative correlation between sulfur concentration, temperature, conductivity, and DO to the density of SOB. This showed that a high density of bacteria caused a lower concentration of sulfur, temperature, conductivity, and DO. The results showed that environmental factors affected the activity of enzymes in bacteria, thereby influencing the efficiency of SOB in treating sulfur (Dong et al., 2023).

3.2. Bacteria community structure based on Oxford Nanopore Technology analysis

Based on the NGS analysis, post-coal mining ponds ≥ 20 years old had 3819 OTUs of bacteria, which was higher than 2027 OTUs of ponds ≤ 5 years old (Figure 4). On the Venn diagram (Figure 4), each circle represented a sample or group of post-coal mining ponds. This showed that age of ponds and physicochemical environmental factors affected the composition and diversity of bacteria. Based on the Venn diagram, there were 1275 OTU in both ages of ponds (≥ 20 years and ≤ 5 years old).

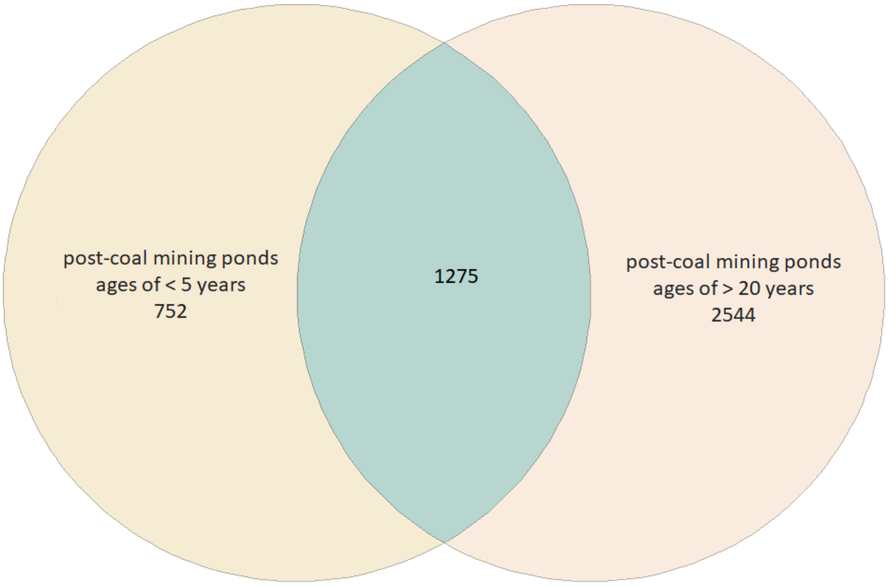


Figure 4. Venn diagram depicting the relationship between sample

The results of the Venn analysis obtained in Figure 4 showed that coal post-mining ponds samples with age of ≥ 20 years had more OTUs when compared to those aged ≤ 5 years. This showed that ponds age and physicochemical environmental factors influenced the composition and diversity of bacteria. In this study, the dominant bacteria phyla in both post-coal mine ponds were Cyanobacteria, Proteobacteria, and Firmicutes (Figure 5). The results were consistent with Wangka et al., (2020), where Proteobacteria, Firmicutes, and Cyanobacteria were the phylum communities found in Bangka Islands sediments based on the results of NGS analysis.

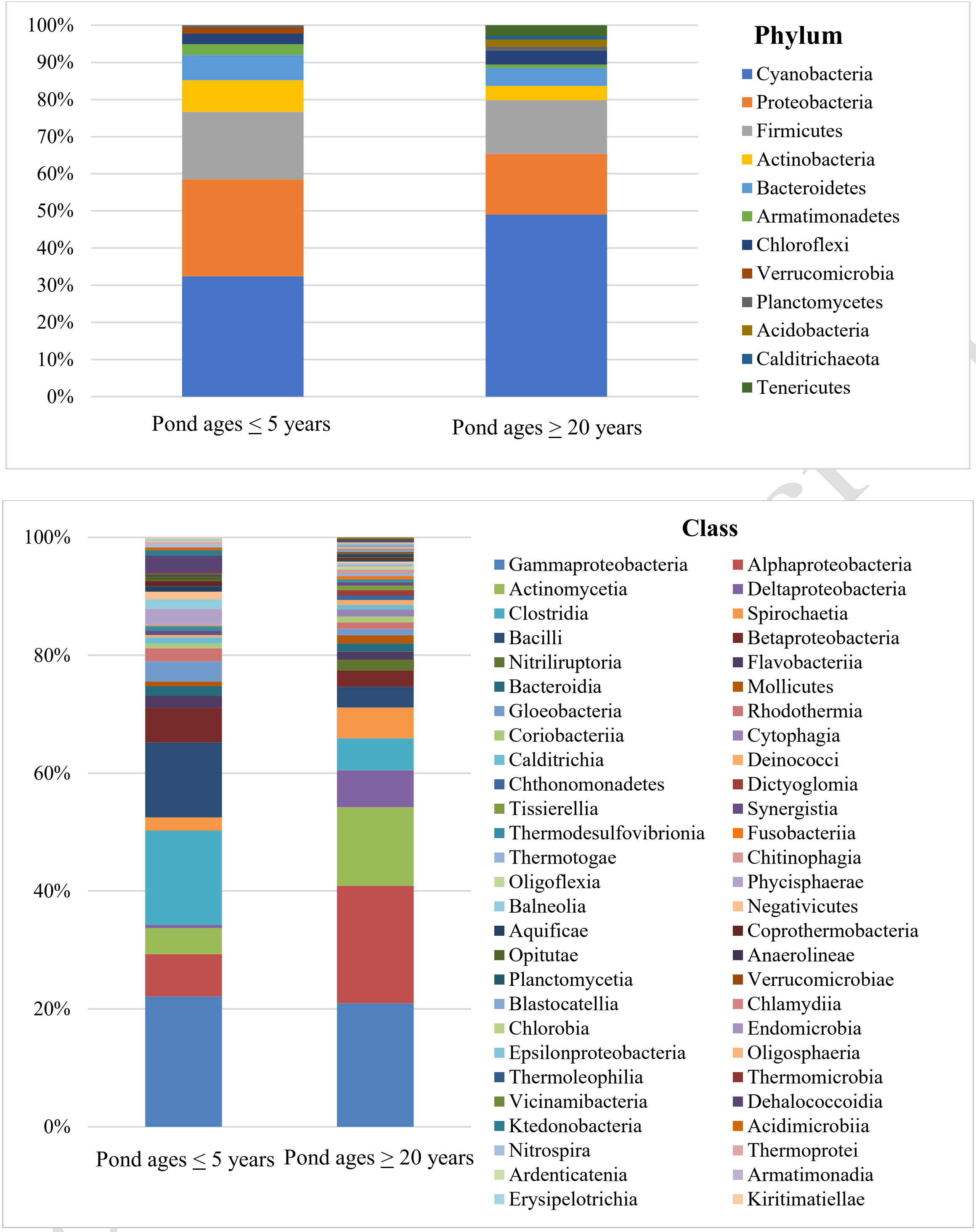


Figure 5. Relative abundant of bacteria phylum and class from post-coal mining ponds ≤ 5 years and ≥ 20 years old

Figure 5 showed that diversity of phyla at ponds ≤ 5 years old consisted of 9 phyla (Cyanobacteria 32%, Proteobacteria 26%, Firmicutes 18%, Actinobacteria 9%, Bacteroidetes 7%, Armatimonadetes 3%, Chloroflexi 3%, Verrucomicrobia 2% and Planctomycetes 1%), while ponds ≥ 20 years consisted of 11 phyla (Cyanobacteria 49%, Proteobacteria 16%, Firmicutes 14%, Actinobacteria 4%, Bacteroidetes 5%, Armatimonadetes 1%, Chloroflexi 4%, Planctomycetes 1%, Acidobacteria 2%, Calditrichaeota 1%, and Tenericutes 3%). The phyla Acidobacteria, Calditrichaeota, and Tenericutes were not found in ponds aged ≤ 5 years, while the phylum Verrucomicrobia was not found in those aged ≥ 20 years. Based on a study conducted by Lee et al. (2009), Verrucomicrobia was a phylum included in the soil and water bacteria community and was typically found in hot springs. Figure 5 also showed diversity of classes in ponds aged ≤ 5 years, where there were 27 bacteria classes, namely Gammaproteobacteria (22%), Clostridia (16%), Bacilli (13%), Alphaproteobacteria (7%), Betaproteobacteria 6%, and others (36%). Class diversity in ponds aged ≥ 20 years consisted of 27 different bacteria classes, comprising Gammaproteobacteria (21%), Alphaproteobacteria (21%), Actinomycetia (13%), Deltaproteobacteria (6%), Clostridia (5%), Spirocahetia (5%), and others (29%).

The phylum Cyanobacteria was found to be dominant in both age groups of coal post-mining ponds. The main role played by Cyanobacteria in ponds ecosystem was that it performed photosynthesis and was the main producer of oxygen. In this process, Cyanobacteria used sulfur and converted it into compounds that could be used by other organisms. This made an important contribution to the sulfur cycle (Kushkevych et al., 2021). Cyanobacteria could also use various nutrients, such as nitrogen, phosphorus, and carbon dioxide in water. The ability of the microbe to use these nutrients helped control nutrient concentrations in ponds, which in turn affected the growth of other organisms in the food chain (Kamennaya et al., 2012).

To determine the relative abundance of genus metagenomically from the 2 ponds ages used as samples in this study, the analysis was carried out and presented in Figure 6.

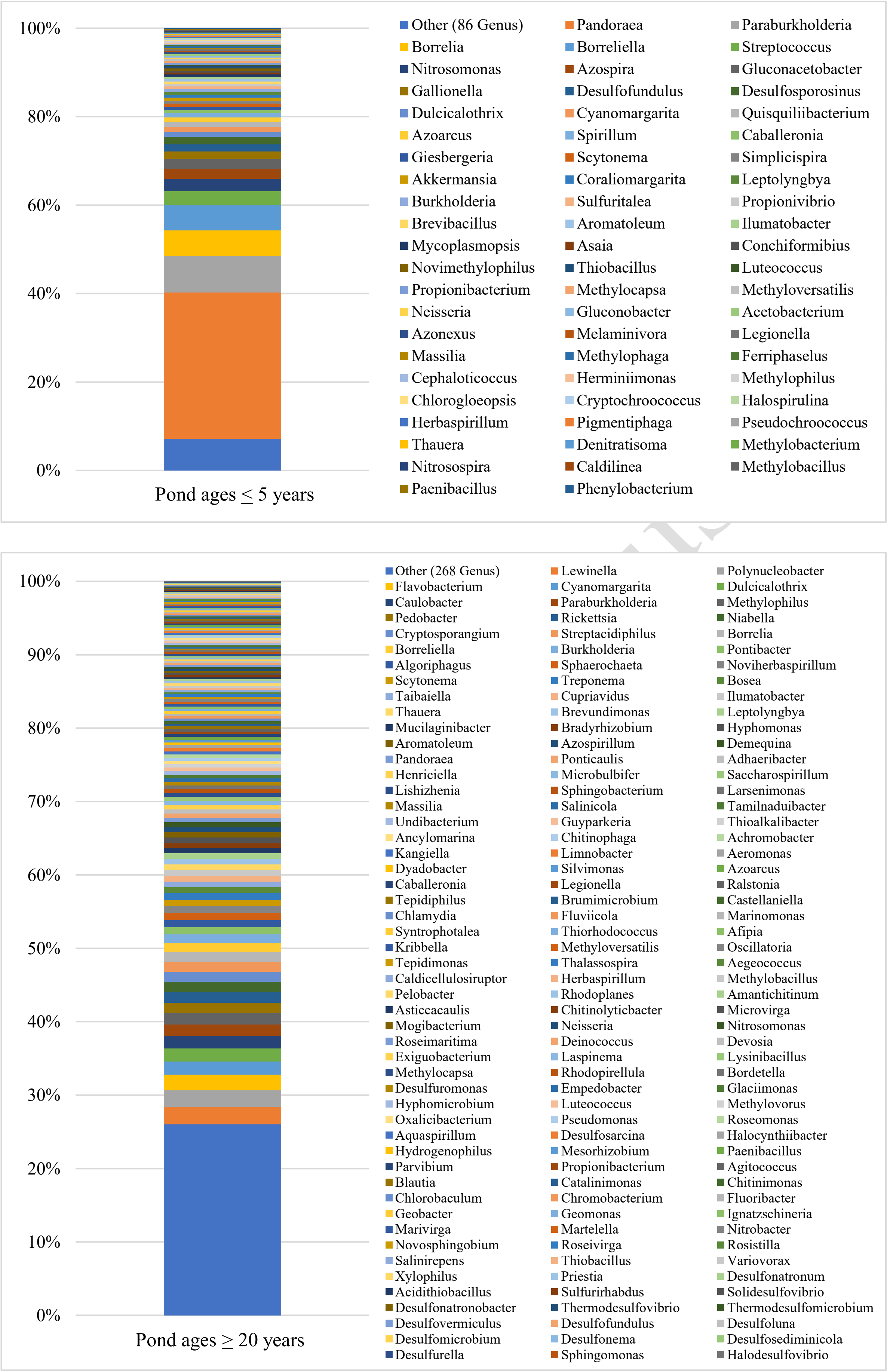


Figure 6. Relative abundant of bacteria genera of two different ages of post-coal mining ponds

Figure 6 showed that bacteria genus richness in ponds ≥ 20 years old was higher compared to ponds ≤ 5 years old. These results showed that ponds age affected bacteria community structure in ponds water. Cole et al., (2019) also showed that bacteria species richness in ponds ≥ 20 years old was significantly higher compared to those aged ≤ 5 years old. This showed that in their activities, SOB were influenced by environmental factors driving the sulfur cycle (Méndez-García et al., 2015; Zhang et al., 2017) and age of post-coal mining ponds because aging caused higher biodiversity (plankton, fish, and other aquatic biota) (Tala'ohu & Irawan, 2014). Diversity and abundance of bacteria in coal-affected environments could be useful bioindicators for post-coal mining ponds restoration. For example, bacteria belonging to the Gammaproteobacteria group could be accurate bioindicators of the biodegradation potential of coal (Akimbekov et al., 2022). Members of the Genus *Acidithiobacillus* were dominantly able to oxidize sulfur in various sulfur-rich environments around the world, and members of this genus were believed to play a key role in the biogeochemical cycles of sulfur. According to the List of Prokaryotic Status in Nomenclature (LPSN) database available at http://www.bacterio.net, several species of the Genus *Acidithiobacillus* had been validated, including *Acidithiobacillus thiooxidans* (*Thiobacillus thiooxidans*), *A. albertensis* (*T. albertis*), *A. caldus* (*T. caldus*), *A. ferrooxidans* (*T. ferrooxidans*), *A. ferrivorans*, *A. ferridurans*, and *A. Ferriphilus* (Wang et al., 2019). *A. thiooxidans* (*T. thiooxidans*) and *A. ferrooxidans* (*T. ferrooxidans*), were generally considered to be the most important bacteria for sulfur oxidation in soil. In this process, sulfur was oxidized and then entered into the biosynthesis of cysteine and methionine (Kushkevych et al., 2020; Melo et al., 2019).

Based on Figure 7, Metagenomic analysis was used to identify the microbial community at post-coal mining ponds ≤ 5 and ≥ 20 years old. The results were helpful in understanding the interaction between microbial communities and the environment.

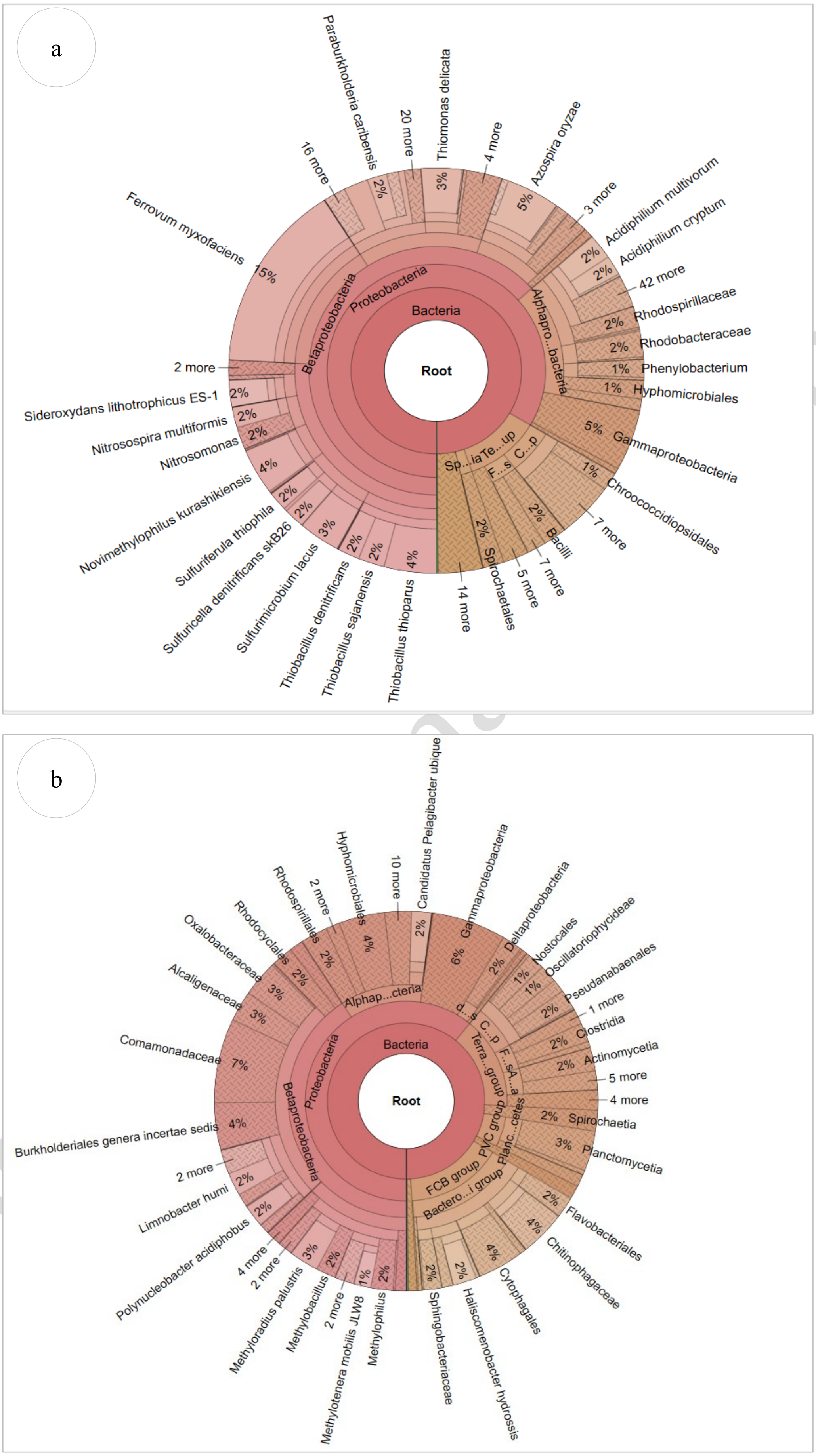
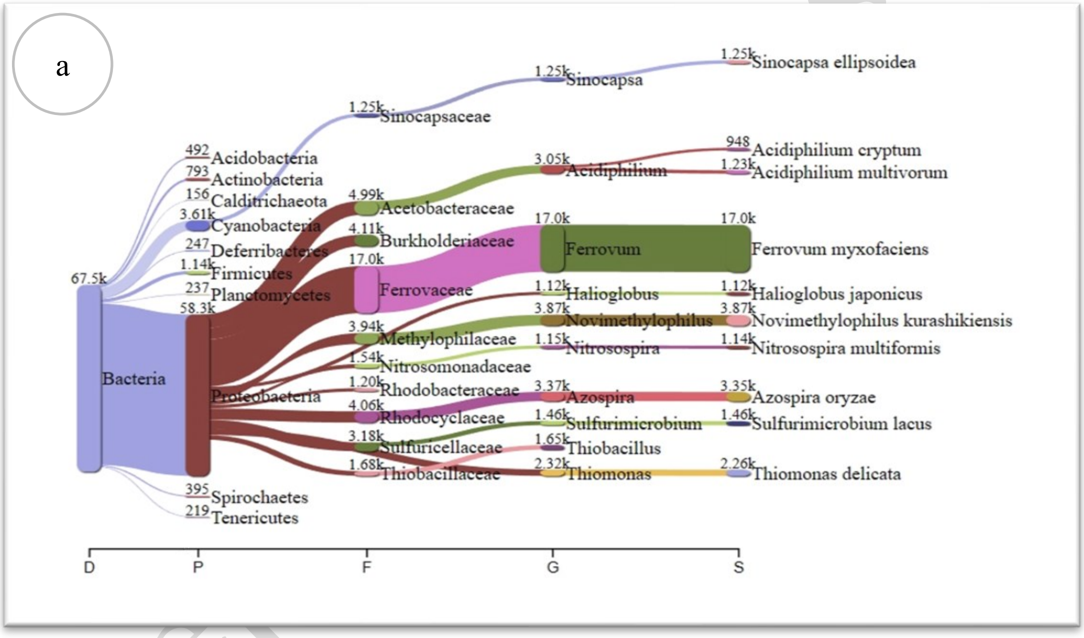


Figure 7. Krona diagram of the relative abundance of bacteria from post-coal mining ponds ≤ 5 years old (a) and ≥ 20 years old (b)

Proteobacteria were a very broad and diverse group of bacteria, which were often found in various environments, including coal mine ponds ecosystems. Among proteobacteria, Betaproteobacteria and Alphaproteobacteria were classes commonly found in highly acidic environments, such as coal post-mining ponds. In addition, coal post-mining ponds tended to have highly acidic conditions due to sulfide oxidation that produced sulfuric acid. Proteobacteria, including Betaproteobacteria and Alphaproteobacteria, had several species that could survive and thrive in these extreme environments. Proteobacteria had the metabolic ability to oxidize compounds found in mining environments, such as sulfur and iron. This allowed them to use the resources available in coal post-mining ponds for their growth. Some species of Proteobacteria, specifically in the Betaproteobacteria class, were engaged in the biogeochemical cycling of minerals, such as sulfur and iron. The microbes could play a role in the oxidation of sulfur and iron compounds commonly present in mining environment, as well as in related chemical processes (Akimbekov et al., 2022).

Sankey Diagram (Figure 8) were used to visualize microbial species of the samples. At post-coal mining ponds ≤ 5 years old for the species level, it was found that the top 10 dominant species were *Sinocapsa ellipsoidea*, *Acidiphilium eryptum*, *Acidiphilium multivorum*, *Ferrovum myxofaciens*, *Halioglobus japonicus*, *Novimenthylophilus kurashikiensis*, *Nitrosospira multiformis*, *Azospira oryzae*, *Sulfurimicrobium lacus*, and *Thiomonas delicata*. The genus Acidiphilium were organisms considered to be acidophilic because of their ability to survive and reproduce in an acidic environment due to contamination by sulfuric acid, such as in post-mining ponds ≤ 5 years old with a value of 2-3 (Stan-Lotter & Fendrihan, 2017). Compared to Acidiphilium, *Ferrovum myxofaciens* were bacteria that were tolerant to highly acidic environments. These microbes could survive in conditions with low pH caused by sulfuric acid resulting from the oxidation of sulfides in coal. In addition, *Ferrovum myxofaciens* was bacteria that oxidized iron and used iron as an energy source. In coal post-mining ponds, there were many oxidized iron minerals and *Ferrovum myxofaciens* could use this as an energy source for their growth (Johnson et al., 2014). At post-coal mining ponds ≥ 20 years old for the species level, it was found that the top 10 dominant species were *Armatimonas rosea*, *Haliscomenobacter* *hydrossis*, *Limnobacter humi*, *Limnobacter thiooxidans*, *Polynucleobacter acidiphobus*, *Methylophilus methylotrophus*, *Methyloradius palustris*, *Methylotenera mobilis*, *Methylotenera versatilis*, and *Candidatus Pelagibacter ubique*. Limnobacter was a genus of bacteria commonly found in aquatic environments, including coal-mining ponds. In addition, Limnobacter were aerobic bacteria, showing the requirement of dissolved oxygen in water to respire. Post-coal mining ponds that were open to the atmosphere could have access to sufficient oxygen to support Limnobacter growth. *Limnobacter thiooxidans* had the ability to oxidize sulfur compounds, such as sulfide and thiosulfate, as an energy source (Spring et al., 2001). In post-coal mining ponds, the sulfur compounds could be present due to the oxidation process of sulfide minerals in coal.



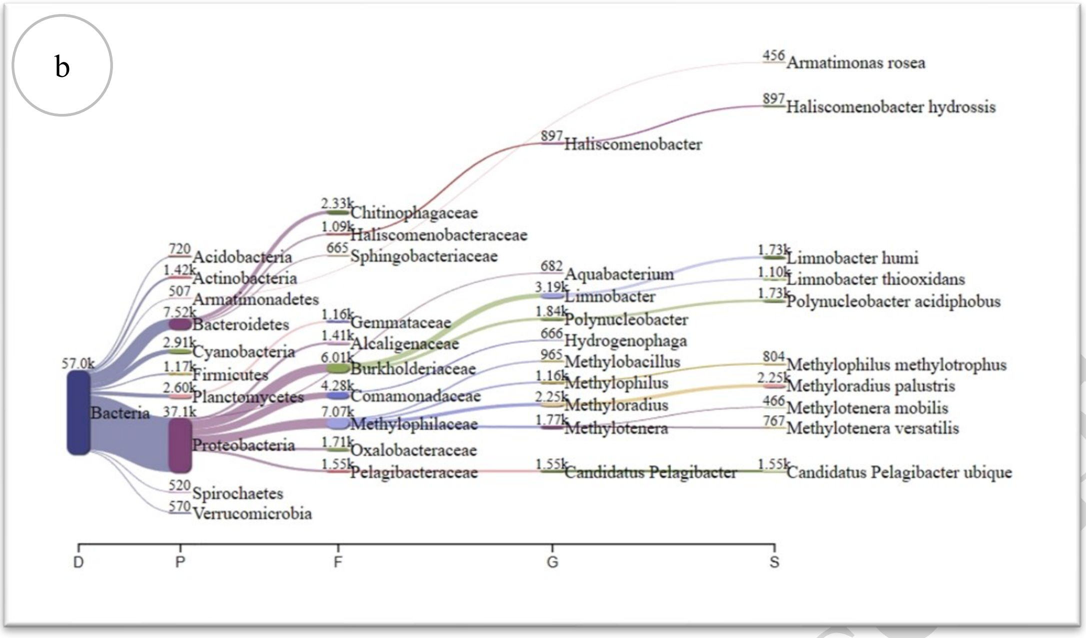


Figure 8. Sankey diagram of the relative abundance of bacteria from post-coal mining ponds ≤ 5 years (a) and ≥ 20 years old (b)

In this study, microbial diversity was determined by the Shannon and Simpson index. The 2 indexes of bacteria diversity provided sample community composition by estimating evenness (Simpson index) and richness (Shannon index). Richness was also measured using 2 types of non-parametric indexes, namely 1) Chao1 to estimate the number of species in community, and 2) ACE to estimate species range. This showed that the number of OTU at post-mining ponds ≥ 20 years old was greater than post-mining ponds ≤ 5 years old. Diversity and richness of bacteria in each post-coal mining ponds showed that diversity at post-mining ponds ≥ 20 years old was higher than at post-mining ponds ≤ 5 years old (Table 2).

Table 2. Bacteria diversity index from post-coal mining ponds water in Samarinda

Sample	Diversity index			
	Chao 1	ACE	Shannon	Simpson
Post-coal mining ponds ≤ 5 years old	3270.47	3301.63	4.18	0.91
Post-coal mining ponds ≥ 20 years old	5449.59	5481.24	6.24	0.99

Based on diversity index in Table 2, the highest diversity was found in post-coal mining ponds ≥ 20 years old. This was influenced by age and physicochemical parameters in post-coal mining ponds. A study conducted by Cole et al., (2019) suggested that ponds age and physicochemical parameters, such as pH and temperature had a significant effect on water quality, abundance of bacteria, and phytoplankton.

4. Conclusion

In conclusion, the results showed that ponds ≥ 20 years old had better water quality and higher bacteria OTU compared to ponds ≤ 5 years old. The richness of phylum, class, family, and genus of bacteria community at post-coal mining ponds ≥ 20 years old was higher compared to ponds ≤ 5 years old. In addition, increasing the abundance of SOB could reduce sulfate concentration of ponds water. The better water quality and higher bacteria community structure at ponds ≥ 20 years old showed the successful remediation of ponds water by SOB.

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