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Water Quality and Microbial Community Assessment in Artisanal Mining- Affected Sediments of Cikidang River, Banten, Java, Indonesia

(Penilaian Kualiti Air dan Komuniti Mikrob dalam Perlombongan Artisanal- Sedimen Terjejas Sungai Cikidang, Banten, Java, Indonesia)

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ABSTRACT

The total microbial diversity and community in submerged sediments near mining sites, transition and intact sites at Cikidang River, Banten were assessed using 16S rDNA sequence data and biodiversity indices. Assessed water quality parameters in the river were water current, dissolved oxygen (DO), pH, redox potential, salinity, temperature and turbidity. Microbial alpha diversity used were Shannon diversity index, ChaoI index and Operational Taxonomic Units (OTUs). These analyses indicated a total of 50 taxa of sediment microbes. Based on Shannon diversity index, the highest value was observed at mining site. High abundant microbes in sediments at mining sites for phylum, class, order, family and genera levels were represented by *Proteobacteria, Gammaproteobacteria, Pseudomonadales, Moraxellaceae,* and *Acinetobacter*, respectively. In contrast, high abundant microbes in the sediment of the intact site for each taxon consisted of *Firmicutes, Alphaproteobacteria, Erysipelotrichales, Erysipelotrichaceae,* and *Erysipelothrix*. The water quality of mining sites was characterised by alkaline pH (8.807, 95% CI: 8.624 - 8.990) and lower redox potential (59.000 mV, 95% CI: 36.233 - 81.767) that differed significantly from the intact sites ($P<0.05$). This study also confirmed that mining sites have a higher genus diversity. Research on potential microbes of related genus as bioremediators could be recommended for further study.

Keywords: Community; microorganism; mining; sediment; 16S rDNA sequence data

ABSTRAK

Seluruh kepelbagaian dan komuniti mikrob dalam sedimen terendam berdekatan dengan tapak perlombongan, peralihan dan lokasi utuh di Sungai Cikidang, Banten telah dinilai menggunakan data jujukan 16S rDNA dan indeks kepelbagaian biologi. Parameter kualiti air yang dinilai di sungai termasuk arus air, oksigen terlarut (DO), pH, potensi redoks, saliniti, suhu dan kekeruhan. Kepelbagaian alfa mikrob yang digunakan adalah indeks kepelbagaian Shannon, indeks ChaoI dan Unit Taksonomi Operasi (OTU). Analisis ini menunjukkan sejumlah 50 taksa mikrob sedimen. Berdasarkan indeks kepelbagaian Shannon, nilai tertinggi diperhatikan di tapak perlombongan. Kelimpahan mikrob yang tinggi dalam sedimen di tapak perlombongan bagi tahap filum, kelas, order, famili dan genus masing-masing diwakili oleh *Proteobacteria, Gammaproteobacteria, Pseudomonadales, Moraxellaceae* dan *Acinetobacter*. Sebaliknya, mikrob yang banyak terdapat dalam sedimen di tapak utuh bagi setiap takson terdiri daripada *Firmicutes, Alphaproteobacteria, Erysipelotrichales, Erysipelotrichaceae* dan *Erysipelothrix.* Kualiti air di tapak perlombongan dicirikan oleh pH alkali (8.807, 95% CI: 8.624-8.990) dan potensi redoks rendah (59.000 mV, 95% CI:36.233-81.767) yang sangat berbeza secara signifikan daripada tapak utuh ($P<0.05$). Kajian ini juga mengesahkan bahawa tapak perlombongan mempunyai kepelbagaian genus yang lebih tinggi. Penyelidikan mengenai mikrob yang berpotensi daripada genus yang berkaitan sebagai bioremediasi boleh disarankan untuk kajian lanjutan.

Kata kunci: Data jujukan 16S rDNA; komuniti; mikroorganisma; perlombongan; sedimen

Introduction

Sediment microbial communities are important natural components for biological activities in river and stream ecosystems. Understanding the microbial dynamics in community structure and function across freshwater environments can provide information on how ecosystems will change in response to nearby anthropogenic land use practices (Gibbons et al. 2014). Sediment microbial communities in river ecosystems are influenced by inputs from nearby ecosystems. Change of dissolved materials in water can cause the change of microbial community in water-sediment habitat.

Artisanal mining is a gold mining method common in river streams. Impacts of artisanal mining on ecosystem and biodiversity have been widely reported which ranged from removal of significant soil volumes to the destruction of massive intact vegetation (de Jesus Pereira 2009; Funoh 2014; Girmay 2018; Macdonald et al. 2015; Meaza et al. 2017). Significant impacts of mining on sediment microbial communities are caused by inputs from sediment runoff. In a contaminated ecosystem like a mining site, high microbial diversity has been observed (Hewson & Fuhrman 2006). Several factors influenced this microbial diversity, including wave, sediment-water content, organic carbon content, chlorophyll a, enzyme activities and inorganic nutrients enrichment (Hewson et al. 2007; Polymenakou et al. 2005; Zhang et al. 2008). However, low microbial diversity has been observed due to a polluted environment. Böer et al. (2009) reported that high nutrient availability reduced diversity because of an increase in competitive exclusion. The competition might influence the distributions of dominant and minor microbial groups in the sediment.

Recent studies of microbial diversity influenced by mining activities have received much attention (Fernandes et al. 2018). Fatimawali et al. (2020) reported a difference in microbial diversity between mining and intact sites. Microbial diversity was observed having either positive or negative correlations with the environmental conditions of mining sites (Deng et al. 2020). Likewise, Basu et al. (2015) observed abundant microbes in former chromium mining sites. While most literature reported microbial diversity in terrestrial sediments, information on microbial diversity in aquatic and submerged sediments are still limited. This information is immediately required regarding the presence of artisanal mining near the river because the river is the source for water supply and irrigation for people living nearby.

Cikidang River is located in Cisungsang village, Cibeber district, Lebak regency, Banten province, Indonesia. This river has high economic and ecological importance, as it provides support for the livelihood of a large number of community members who are mainly farmers. Recently, this river was threatened by artisanal mining activities and an area of 6445 Ha had been occupied by mining activities as reported by Kurniawan et al. (2013). In contaminated rivers, nutrients and hazardous pollutants exist simultaneously in the sediment. Likewise, few studies have been conducted to investigate the microbial communities in contaminated river sediments related to the artisanal mining activities nearby. Here, the objectives of this study were to explore the profile of total sediment microbial diversity in contaminated river sediment adjacent to the mining sites. The microbial diversity was assessed using 16S rDNA sequence data.

Materials and Methods

Study area

The study was conducted on July 2020 in 3 stations located at Cikidang River, Banten Province (Figure 1). Those stations were selected considering the distances to the gold mining sites that included near the mining site (station A), transition (station B) and intact sites (station C) located far from the mining sites. Transition was a site that consisted of combinations of mining sites and agricultural lands. Intact site was a site that consisted only agricultural lands without any mining activities. Each sampling station and mining site geocoordinates in decimal degrees were recorded using Global Positioning System (GPS) handheld Etrex Garmin. All sampling stations were situated at latitude of 6.791556 S-6.816861 S and longitude of 106.420389 E- 106.438722 E. Total distance of all sampling stations was 4 km covering Cikidang River with a width of 20 m.

Microbial sediment sampling

Microbial sediment sampling (Adibe et al. 2020; dos Santos Furtado & Casper 2000; Zhang et al. 2019) was based on triplicate sediment cores collected from the designated sampling stations using a core sampler. In each core, approximately 10 g sediments were collected at a depth of about 5 cm from the sediment surface using a small shovel. The sediment samples were maintained at ± 30 °C and then transported for further microbial analysis using 16S rDNA sequence data.

WATER QUALITY MEASUREMENT

Water quality data from each station were collected simultaneously (*in situ*) with sediment samplings with 3 replicates. The measured parameters included water current, dissolved oxygen (DO), pH, redox potential, salinity, temperature, and turbidity. Water current was measured using Flowatch FL 03, DO and temperature were measured using DO meter (Lutron DO 5510), redox potential and pH were measured using pH meter (Lutron PH 208), salinity was measured using a refractometer (Atago) and turbidity was measured using turbidity meter (Ezdo TUB-430).

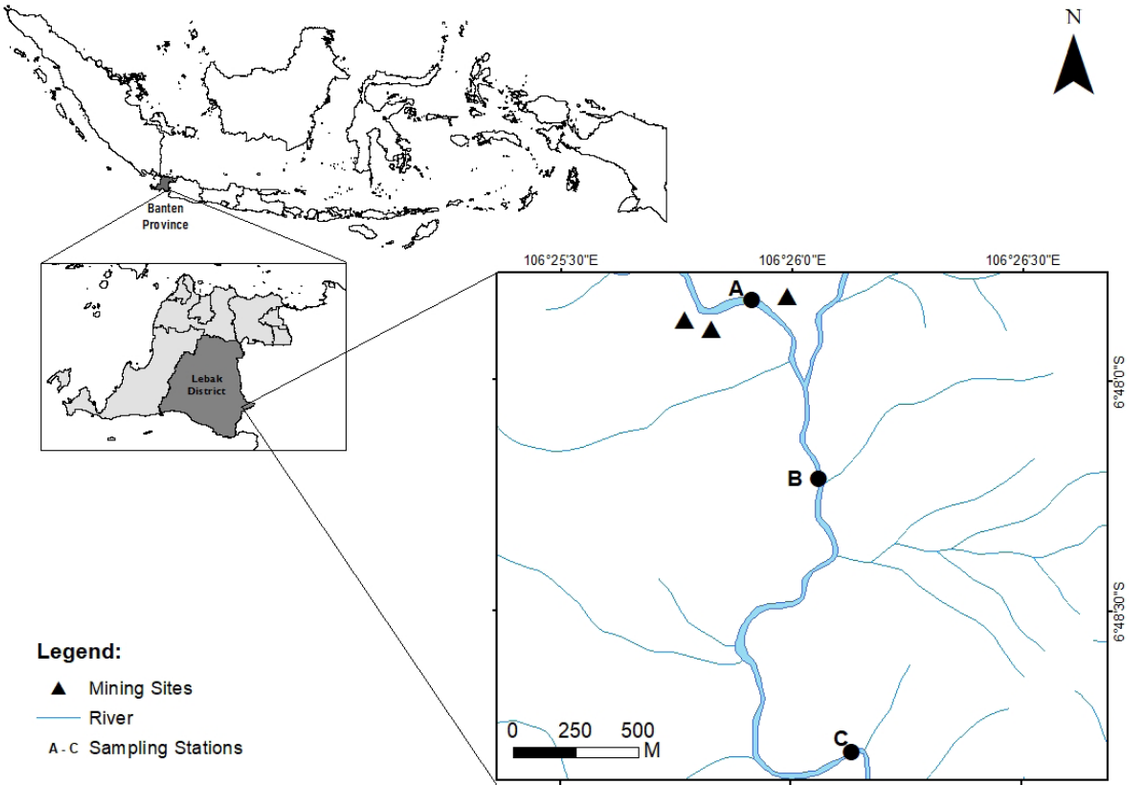


FIGURE 1. Location of Cikidang River indicating the sampling sites of points A, B, C and the mining sites

EXTRACTION OF DNA SAMPLES AND AMPLICON SEQUENCING

Sediment samples were sent to PT Genetika Science Indonesia for DNA extraction. The extraction was perfomed using ZymoBIOMICS™ DNA Miniprep Kit following the manufacturer's instructions (Abundo et al. 2021). The gDNA samples were then sent to Novogene Bioinformatics Technology Co., Ltd (Singapore) for amplicon sequencing. The bacterial 16S rDNA was amplified using specific primer 16S V3-V4 (341 F – 806 R) 5' CCTAYGGGRBGCASCAG – 3' GGACTACNNGGGTATCTAAT. Samples with bright main fragments between 400 and 450bp were chosen for further experiments. PCR products were mixed at equal density ratios. The mixed PCR products were purified with Qiagen Gel Extraction Kit (Qiagen, Germany). The libraries were generated using NEBNext® Ultra™ DNA Library Prep Kit for Illumina and quantified via Qubit and QPCR, was analysed by Illumina platform. The procedure was adapted from previous study (Amelia et al. 2020; Fatimawali et al. 2020; Ke et al. 2019; Navitasari et al. 2020; Zhang et al. 2021).

BIOINFORMATICS ANALYSIS

Bioinformatics analysis were conducted by Novogene Bioinformatics Technology Co., Ltd (Singapore). Paired-end reads were assigned to samples based on their unique barcodes and truncated by cutting off the barcode and primer sequences. Paired-end reads were merged using FLASH (V1.2.7 (V1.2.7,http://ccb.jhu.edu/software/FLASH/) (Magoč & Salzberg 2011). The tags were compared with the reference database (Gold database, http://drive5.com/uchime/uchime_download.html) using UCHIME algorithm (UCHIME Algorithm, http://www.drive5.com/usearch/manual/uchime_algo.html) (Edgar et al. 2011) to detect chimeric sequences, and then the chimeric sequences were removed (Haas et al. 2011). Effective tags were finally obtained. Sequence analysis was performed using Uparse software (Uparse v7.0.1001, see details http://drive5.com/uparse/) (Edgar 2013) using all the effective tags. Sequences with ≥97% similarity were assigned to the same Operational Taxonomic Units (OTUs). Representative sequence for each OTU was screened for further annotation. For each representative sequence, Mothur software was performed against the SSUrRNA database of SILVA Database (see details http://www.arb-silva.de/) for species annotation at each taxonomic rank (Threshold:0.8~1) (kingdom, phylum, class, order, family, genus, species) (Navitasari et al. 2020). To obtain the phylogenetic relationship of all OTUs representative sequences, the MUSCLE (Version 3.8.31, http://www.drive5.com/muscle/) was used to compare multiple sequences rapidly (Quast et al. 2013). OTUs abundance information was normalised using a standard of sequence number corresponding to the sample with the least sequences. Subsequent analysis of alpha diversity and beta diversity were all performed basing on this output normalised data. Alpha diversity Chao1 and Shannon in the samples were calculated with QIIME (Version 1.7.0) and displayed with R software (Version 2.15.3) (Amelia et al. 2020).

STATISTICAL ANALYSIS

The microbial diversity was calculated using Shannon index. All water quality data were presented in mean and 95% CI. The differences of water quality data among stations were calculated using one-way ANOVA and post hoc test (Navitasari et al. 2020). The significance of data differences was determined at $P < 0.05$.

RESULTS AND DISCUSSION

WATER QUALITY

The mean and 95% CI of water quality parameters in sampling stations A, B and C were as presented in Table 1. Station A was located upstream and near the mining site. Stations B and C were located downstream and far from the mining site, respectively. These stations were classified as transition and intact sites, respectively. Water quality is an important variable for the presence of microbes in sediment since the sediments were submerged in water (Shukla et al. 2017). The water current was similar between mining site, transition and intact sites (Table 1). DO was higher in mining site than

TABLE 1. Mean and 95% CI of water quality variables in sampling stations A, B and C

Variables	Station A	Station B	Station C	P	F
Current (ms^{-1})	3.033[a] (-2.066-8.132)	3.467[a] (-1.130-8.063)	2.433[a] (-2.832-7.699)	0.824	0.200
DO (mgL^{-1})	8.500 [a] (8.252-8.748)	7.867[b] (7.487-8.246)	7.933 [b] (7.790-8.077)	0.001	29.727
pH	8.807[a] (8.624-8.990)	9.163[b] (9.101-9.226)	8.233[c] (7.854-8.613)	0.000	67.389
Redox potential (mV)	59.000[a] (36.233-81.767)	78.667 [b] (74.872-82.461)	68.000[b] (61.428-74.572)	0.014	9.346
Salinity (ppt)	4.667[a] (3.232-6.101)	5.333 [a] (3.899-6.768)	6.66[b] (5.232-8.101)	0.014	9.333
Temperature (°C)	22.633[a] (22.008-23.258)	23.867[b] (23.487-24.246)	24.500[c] (24.003-24.997)	0.000	64.003
Turbidity (NTU)	4.197[a] (-3.803-12.197)	6.447[a] (2.513-10.380)	97.900 [b] (-23.558-219.358)	0.010	10.703

[a,b,c]: means with different superscript letters in the same row indicated significant differences by post hoc test ($p < 0.05$). P value with significance level < 0.05; F is value of ratio of 2 different measure of variance for the data

other sites ($P < 0.005$). The highest mean DO was 8.500 mgL^{-1} and the lowest was 7.867 mgL^{-1}. The transition site has the highest pH with a value of 9.163 ($P < 0.005$). Salinity was higher ($P < 0.005$) in the intact site that was located downstream. Mining site located in the upstream has lower salinity. The water in transition and intact sites in the downstream areas was warmer ($P < 0.005$) than the mining site in upstream areas. The water of transition and intact sites downstream were also more turbid than upstream ($P < 0.005$). However, station A located near the mining site had a lower redox potential value than the transition and intact site in stations B and C ($P < 0.005$).

SEDIMENT MICROBIAL COMMUNITY

The composition of sediment microbial communities in 3 different environmental conditions derived from a near mining site (Station A), transition site (Station B) and intact site (Station B) were assessed and compared using 16S rRNA gene sequence. Figure 2 shows the rarefaction curves generated at 1% cutoff to make a comparison of microbial species richness among sites. Higher OTUs indices of sediment microbial communities near mining sites than transition and intact sites (Figure 2) were observed in this study which demonstrated the usefulness of 16S rDNA sequence in revealing the variation of microbial diversity influenced by artisanal mining. Based on OTUs and ChaoI indices of microbial richness, it was apparent that Station A displayed relatively higher species richness, followed by Stations B and C (Figure 3). Likewise, the Shannon diversity indices showed similar trends with the highest values observed for samples from station A (7.111), when compared with those from stations B and C (6.756 and 6.636).

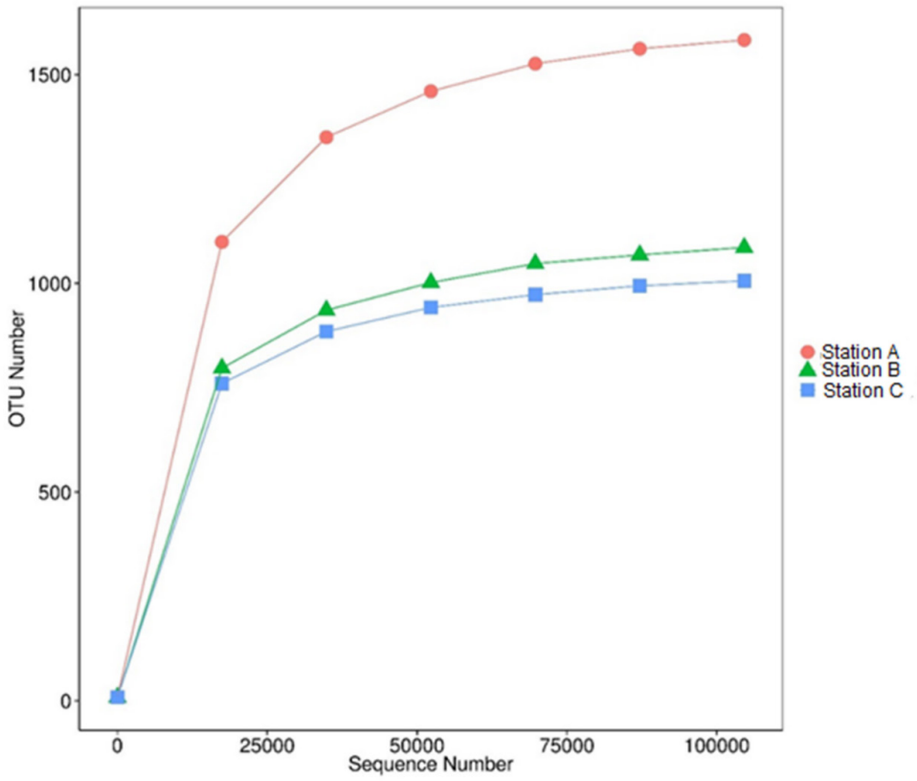


FIGURE 2. Rarefaction curves showing the observed number of operational taxonomic units (OTUs) at 1% dissimilarity for sediment microbial samples from sampling stations A (round-redline), B (triangle-greenline) and C (square-blueline)

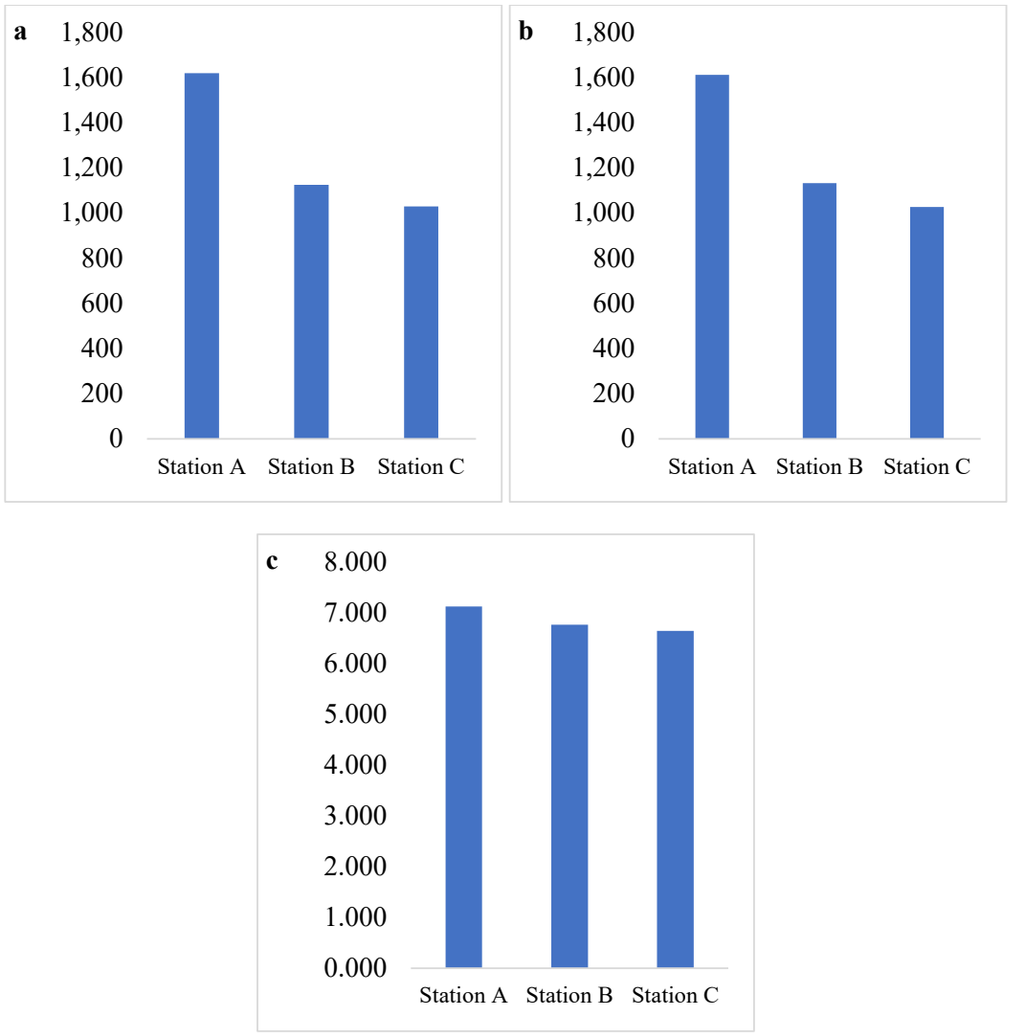
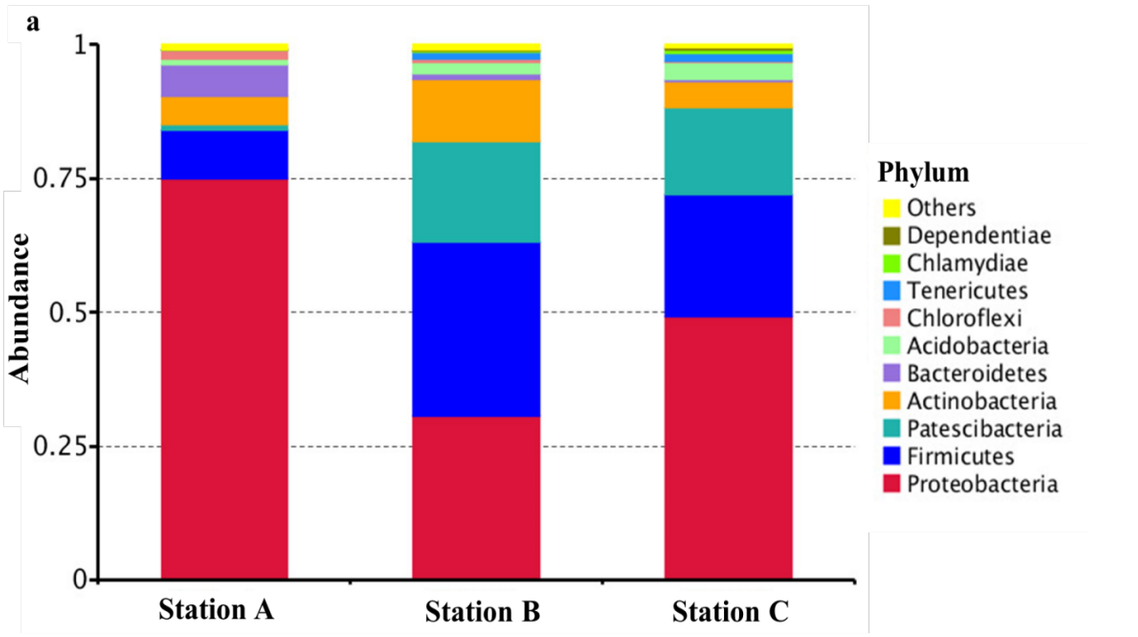


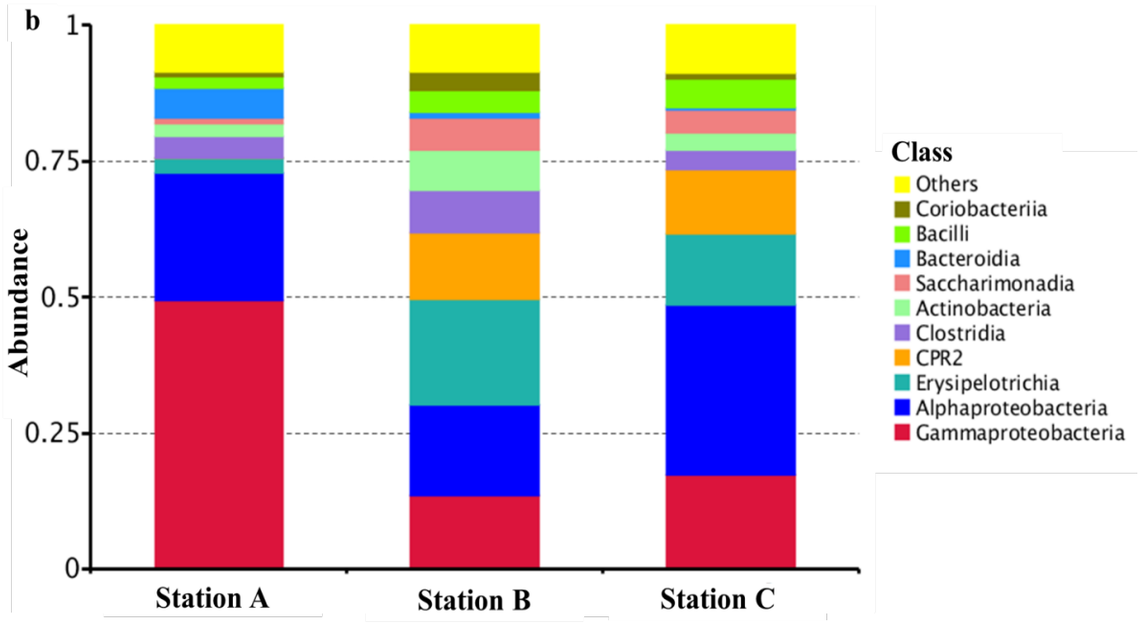
FIGURE 3. Aggregated richness indices at a genetic distance of 1% represented as the number of observed OTUs (A), ChaoI (B) and Shannon Index (C)

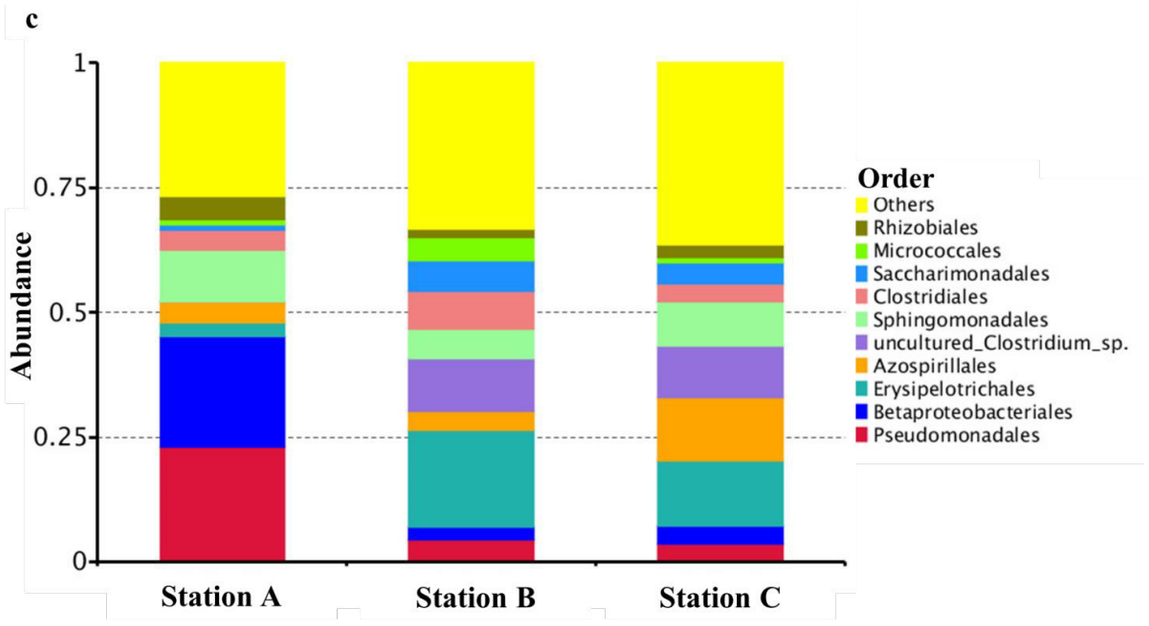
Figure 4(a) showed the phylum compositions among sites. The mining site (Station A) was mainly dominated by *Proteobacteria* (75%) followed by *Firmicutes, Actinobacteria,* and *Bacteroidetes*. Transition site (Station B) had a more proportional abundance among *Proteobacteria, Firmicutes, Actinobacteria,* and *Patescibacteria. Acidobacteria* in mining site were increased in transition site. A similar pattern was also observed in the intact site (Station C) that was dominated by *Proteobacteria* and followed by *Firmicutes, Actinobacteria,* and *Patescibacteria*. *Bacteriodetes* were lower in stations B and C. The most apparent abundance at the phylum level was belonging to *Proteobacteria*. There were several phyla observed in low abundance which included *Chlamydiae, Chloroflexi, Dependentiae,* and *Tenericutes.*

A dominance of particular taxa was also observed in microbial diversity at the class level (Figure 4(b)). In the mining site, *Gammaproteobacteria* accounted for half (50%) of abundance followed by *Alphaproteobacteria* (25%). In the transition and intact sites, *Gammaproteobacteria* abundance decreased. *Erysipelotrichia* dominated the transition site followed by *Alphaproteobacteria, Gammaproteobacteria,*

Clostridia, Actinobacteria, and *Sacharimonadia*. The intact site was dominated by *Alphaproteobacteria* and followed by *Gammaproteobacteria* and *Erysipelotrichia*. At order level (Figure 4(c)) in mining site, *Pseudomonadales* and *Betaproteobacteriales* dominated the microbial diversity and followed by low abundance from classes of *Erysipelotrichales, Azospirillales, Sphingomonadales,* and *Clostridiales*. Nonetheless, in transition and intact sites, the abundance of *Pseudomonadales* and *Betaproteobacteriales* decreased and replaced by the dominance of *Erysipelotrichales* and *Azospirillales*.



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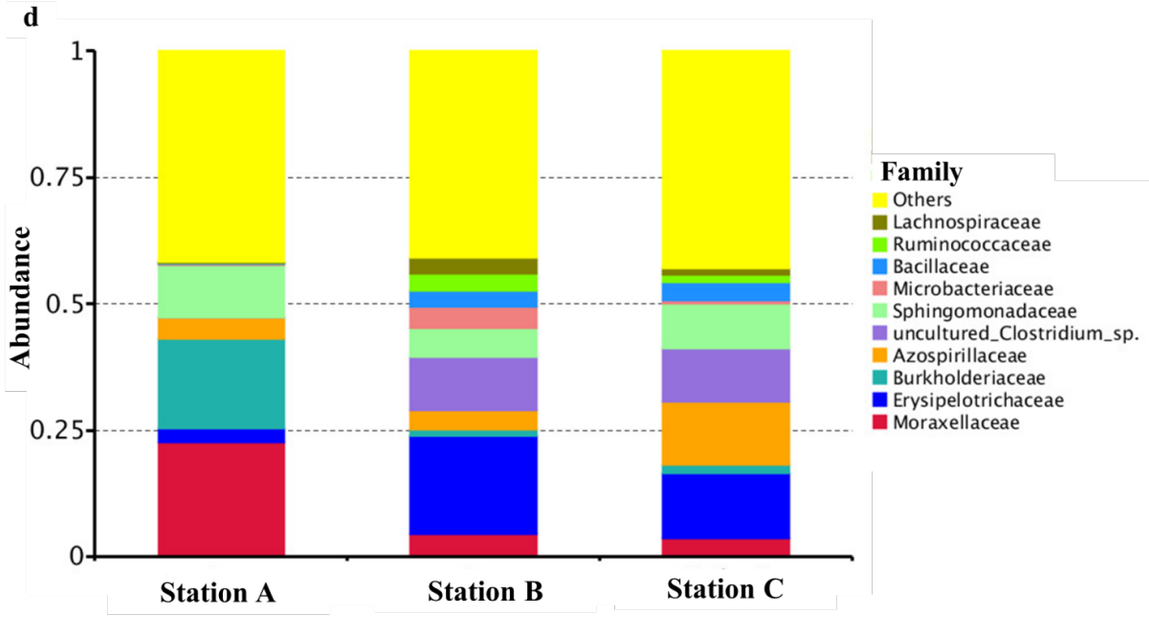
Betaproteobacteriales
Pseudomonadales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Ruminococcaceae
Bacillaceae
Microbacteriaceae
Sphingomonadaceae
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Azospirillaceae
Burkholderiaceae
Erysipelotrichaceae
Moraxellaceae

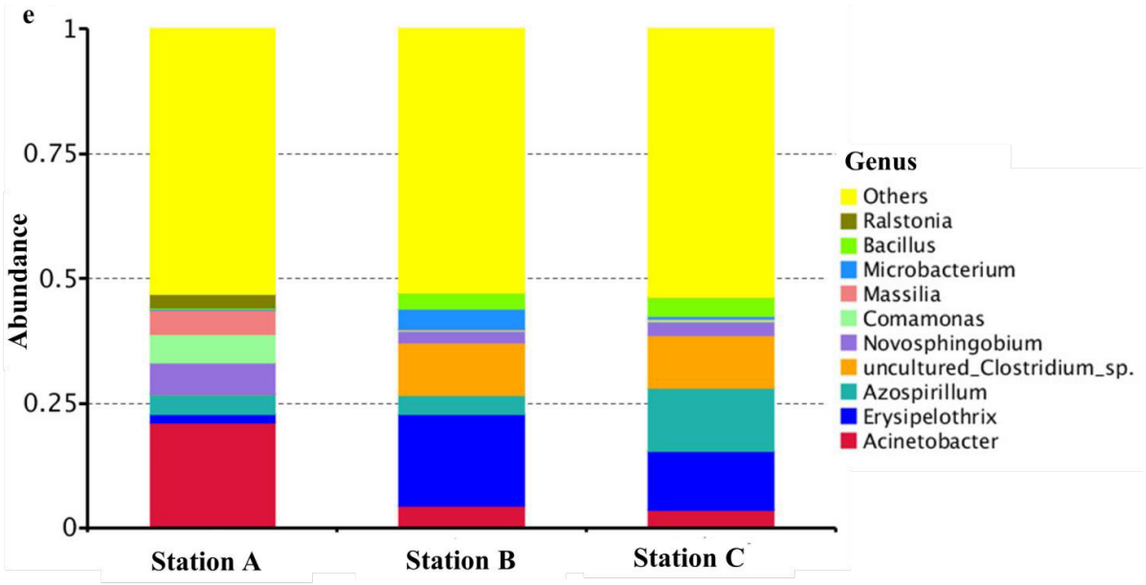

FIGURE 4. Microbial abundance at phylum (a), class (b), order (c), family (d) and genus (e) levels in sediments from sampling stations A (mining site), B (transition site) and C (intact site)

At family level (Figure 4(d)) in mining site, *Moraxellaceae, Burkholderiaceae,* and *Sphingomonadaceae* were the dominant microbial family. The other families with low abundance were *Erysipelotrichaceae* and *Azospirillaceae*. In transition and intact sites, *Moraxellaceae* abundance decreased and was replaced by *Erysipelotrichaceae*. In those sites, there was a presence of some families that were not observed in mining site. Those families included *Lachnospiraceae, Ruminococcaceae, Bacillaceae, Microbacteriaceae,* and *Clostridium.*

Figure 4(e) presented the abundance of microbial species at the genus level. Abundances of some genera were observed increasing and decreasing. Mining site was dominated by *Acinetobacter* that decreased in transition and intact sites. *Erysipelothrix* and *Azospirillum* abundances that were low in mining sites increased in transition and intact sites. *Novosphingobium* that was high in mining site was found to decrease in transition and intact sites. However, microbial diversity in the mining site was higher since *Ralstonia* and *Massilia* were also present there.

Figure 5 showed the 16S rRNA gene-based phylogeny representatives of all microbial genera from stations near mining, in transition site and intact site far from mining sites. It was apparent that station A near mining sites was dominated by *Acinetobacter*, *Comamonas*, *Massilia*, *Novosphingobium,* and *Azospirillum*. High abundant genera in station B consisted of *Acinetobacter*, *Azospirillum*, *Novosphingobium,* and *Sphingomonas*. In station C, the genus with the highest abundance was *Azospirillum* followed by *Novosphingobium* and *Sphingomonas*.

DISCUSSION

Variation in water quality parameters can be related either to natural factors or anthropogenic influences. Natural influences can be seen in salinity values that were higher in Station C. This station was located in the downstream areas. In upstream areas in Station A, the DO was recorded higher than in other areas. This station was located in upstream areas with an altitude higher than other stations. Differences in altitude have caused water flow from high to low altitude in downstream and this causes water turbulence. The more turbulence that a stream or river displays (Atapaththu et al. 2017), then more oxygen are absorbed into the water and increases DO. Higher turbidity observed in stations far from mining sites was related to the actual land uses. The mining sites were located deep in the forest with the surrounding land uses were closed canopy vegetation without anthropogenic influences whereas Stations B and C were located near the residential and agricultural land uses. The

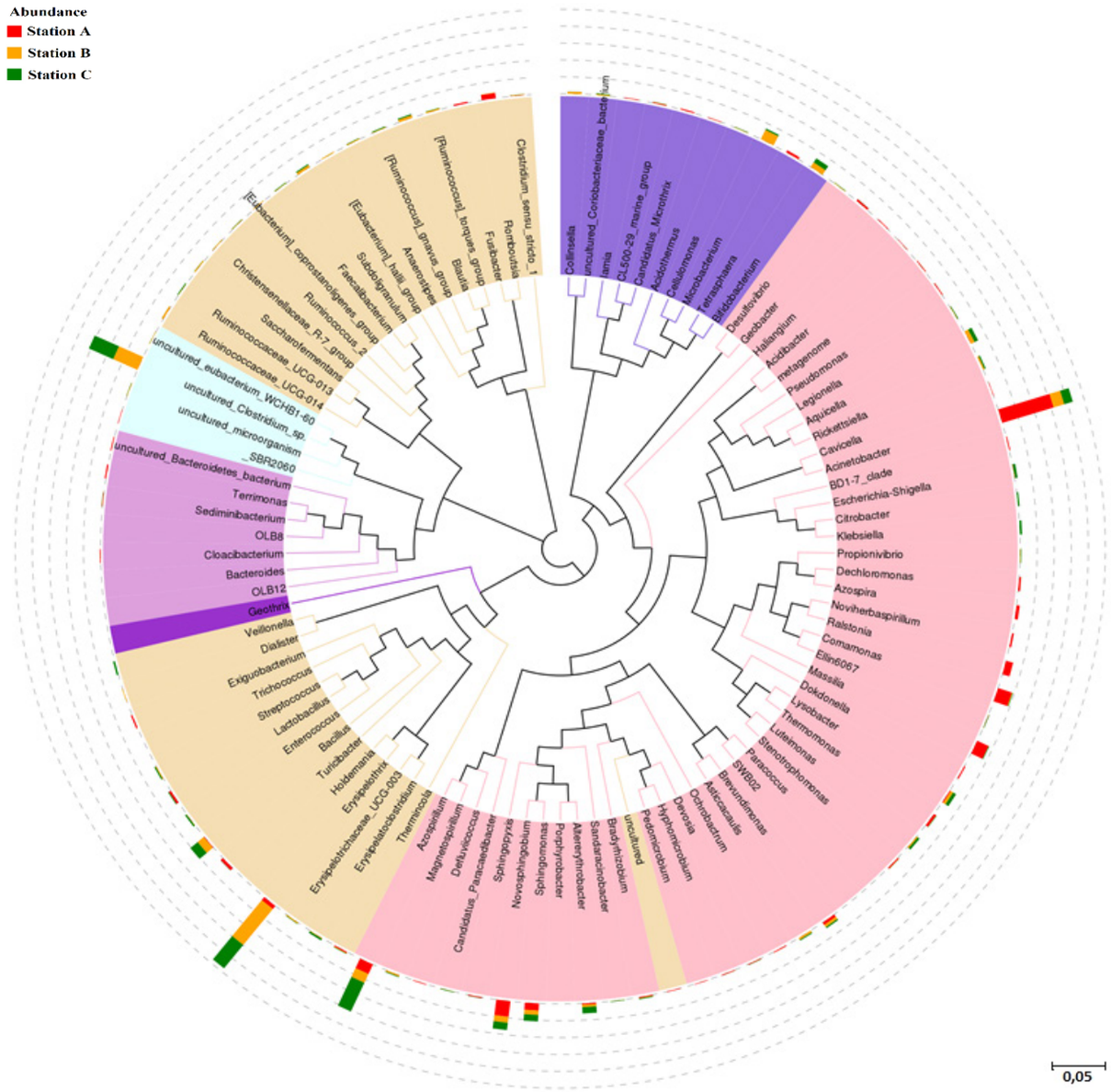

FIGURE 5. 16S rRNAgene-based phylogeny showing representatives of all microbial genera in sediments from stations A (red bar), B (orange bar) and C (green bar). Molecular phylogenetic analysis by maximum likelihood method (scale bar 0.05)

agricultural practices in the forms of paddy field and swidden agriculture have removed the vegetation covers that obstruct and limit the surface runoff. Without the presence of vegetation covers, materials from surface run off and agricultural land uses were entering the river and increasing the turbidity. The impacts of mining site were apparent and could be seen in the redox potential values. The sampling station located near the mining sites was apparently having low redox value. This condition was corroborated with the results from Bachmann et al. (2001) and Campaner et al. (2014). In their study, the redox potential value near the mining sites was as low as 445 mV while the value for the intact site was as high as 625 mV. The range of pH (7.854 - 9.226) in this study

was higher compared to Gafur et al. (2018) with the range of 7.53 to 7.8 and Martín et al. (2020) in range of 7.56 to 7.63. The higher range of pH could be caused by tailing process in gold mining. He et al. (2014) stated that pH in tailing could reach alkaline level (pH = 9).

In terms of microbial diversity, rarefaction curves indicated ChaoI, OTUs, and Shannon diversity reached saturation in all samples. The Shannon index, a measure of richness and evenness, was statistically higher for station near mining sites. In this study, microbial diversity in river communities was assessed using the 16S rDNA sequence data approach. One of the significant advantages of using targeted metagenomic techniques including 16S rRNA gene sequencing, is because they are culture-independent and can theoretically recover almost all microbial taxa in any habitat including polluted ecosystem (Locey & Lennon 2016). The variations of microbial diversity at various taxa levels in sediments as observed in this study were related to the surrounding water quality. Sheaves et al. (2018) stated that degraded water quality caused by pesticide and fertiliser uses, mill tailings and village activities around the stream can alter the water quality in water and sediment.

In our study, Phylum *Proteobacteria, Firmicutes,* and *Actinobacteria* are abundant in all sites. This finding in our study was in accordance with Chodak et al. (2015), Fatimawali et al. (2020), Ji et al. (2018), and Li et al. (2021). Ji et al. (2018) reported that these phyla have high proportion in contaminated area such as mining which indicated high tolerancy to heavy metal.

In this study, it could be said that the microbial composition among locations were varied. *Acinetobacter* presence near mining site was related to the preference and adaptation of this genus. *Acinetobacte*r is also known as ubiquitous bacteria which is capable in degrading a variety of organic pollutants (Bergogne-Bérézin 2014). Anderson and Cook (2004) explained that *Acinetobacter* has the capability to reduce arsenate to arsenite using a non-respiratory mechanism and this explained the preference of these genera on gold mining sites that might contain arsenic (Cai et al. 2017; Eisler 2004). In addition, there were three genera that were only found at the mining site (A). Those genera were *Ralstonia*, *Massila*, and *Commamonas*. *Rasltonia* is a genus known to be applied as bioreactor in metal removal through precipitation (Batt 2014). One of the species of *Commamonas* namely *C. testosteroni* was reported to be found in placer gold deposits (Melchiorre et al. 2018), while genus *Massilia* in subsurface soil of a metal mine (Du et al. 2012). Genera *Erysipelothrix* and *Clostridium* were found to be abundant in site B and site C. Genus *Erysipelothrix* was found in sulfate-rich environments, in association with SO_4^{-2} reducers and is potentially involved in metal and H redox (Hatam et al. 2019). Some researchers reported that the Genus *Clostridium* had the ability to reduce and precipitate metals such as europium (Eu) (Maleke et al. 2019), palladium (Pd) (Chidambaram et al. 2010) and copper (Cu) (Hofacker et al. 2015). Meanwhile, genus *Microbacterium* that was only found in transition site (B) had been found in various environmental sources such as soil and water samples (Chorost et al. 2018).

Related to ecological and health impact, several genera which were in abundance such as *Acinetobacter*, *Erysipelothrix*, *Azospirillium*, *Clostridium,* and *Novosphingobium* collected from the study site had been widely reported to have a role in nutrient cycle or organic decomposer as well as disease agents. *Acinetobacter* are saprophytic bacteria and can be found in soil and water (Bergogne-Bérézin 2014). This genus has species that cause diseases such as pneumonia (Poduch & Kotra 2007). *Clostridium* is capable of nitrogen fixation as a plant nutrient and the production of compounds such as biohydrogen and acetone (Figueiredo et al. 2020). This genus had also been found in various habitats including rivers (Samanta & Bandyopadhyay 2020). *Clostridium* are anaerobic, fermentative, spore-forming Gram-positive bacteria belonging to the phylum *Firmicutes* (Bowman 2011). One of the species from genus *Erysipelothrix* namely *E. piscisicarius* is known as pathogenic bacteria that causes infection in fish (Pomaranski et al. 2020). *Azospirillium* is known as a nitrogen fixing bacteria. These bacteria are important for soil fertility and growth (Fukami et al. 2016; Steenhoudt & Vanderleyden 2000). *Novosphongobium* is known to be widely distributed, including in contaminated environments. This genus has the ability to degrade xenobiotic compounds (Wang et al. 2018).

Conclusions

The most abundant genus in mining site (A) was *Acinetobacter*, in transition site (B) was genus *Erysipelothrix* and in intact site (C) were genus *Erysipelothrix*, genus *Azospirillum* and genus *Clostridium*. Meanwhile, *Comamonas*, *Massilia,* and *Ralstonia* were only found in the mining site (A). In the transition site there was *Microbacterium* which was not found in other sites. Our study showed that the mining

site displayed high richness in genus members compared to other sites. Further research is needed to investigate the genus that can be used as bioremediation agent.

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